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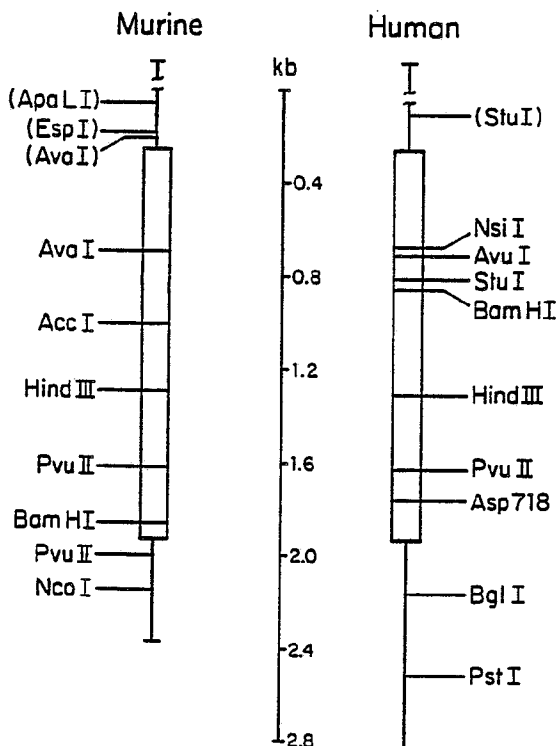
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(54) Title: INTERLEUKIN-1 RECEPTORS



(57) Abstract

Mammalian Interleukin-1 receptor proteins (IL-1Rs), DNAs and expression vectors encoding mammalian IL-1Rs, and processes for producing mammalian IL-1Rs as products of cell culture, including recombinant systems, are disclosed.

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TITLE

Interleukin-1 Receptors

BACKGROUND OF THE INVENTION

5 The present invention relates generally to cytokine receptors, and more specifically, to Interleukin-1 receptors.

 Interleukin-1 α and Interleukin-1 β (IL-1 α and IL-1 β) are
distantly related polypeptide hormones which play a central role in
the regulation of immune and inflammatory responses. These two
10 proteins were originally both classified as IL-1, based on a shared
lymphocyte activation factor (LAF) activity, and a common major
cellular source, activated macrophages. As information has
accumulated from studies using purified natural and recombinant IL-1
molecules, it has become clear that IL-1 α and IL-1 β each mediate most,
15 if not all, of the wide range of activities previously ascribed to
IL-1. The basis for this nearly identical spectrum of biological
activities is thought to be a single class of plasma membrane IL-1
receptors which bind both IL-1 α and IL-1 β .

 A few preliminary reports concerning the existence of an IL-1
20 plasma membrane receptor have been published. To date, structural
characterization of the Interleukin-1 receptor has been limited to
estimates of the molecular weight of this protein by gel filtration,
by SDS-PAGE analysis of covalent complexes formed by chemical
crosslinking between the receptor and 125 I-IL-1 molecules, and by
25 immunoprecipitation of labeled surface proteins.

 Dower et al. (J. Exp. Med. 162:501, 1985), and Dower et al.
(Proc. Natl. Acad. Sci. USA 83:1060, 1986), describe chemical
crosslinking studies indicating an apparent 79.5 kilodalton (kDa)
plasma membrane protein on LBRM-33-1A5 murine T lymphoma cells and a
30 78 kDa surface protein on a murine fibroblast cell line which bound
 125 I-labeled human Interleukin-1 β . Kilian et al. (J. Immunol.
136:4509, 1986) reported that murine 125 I-IL-1 α binding to murine
thymoma cells could be blocked by human IL-1 α and IL-1 β . Dower et al.
(Nature 324:266, 1986) reported binding competition studies indicating
35 that IL-1 α and IL-1 β bound to the same cell surface receptors on

LBRM-33-1A5 cells, human dermal fibroblasts, murine BALB-3T3 cells, and ARH77, a human B lymphoblastoid cell line. The receptors in the different cell lineages exhibited similar but not identical binding characteristics. The IL-1 receptors on porcine synovial fibroblasts (Bird et al., Nature 324:263, 1986) and human dermal fibroblasts (Chin et al., J. Exp. Med. 165:70, 1987) have been shown to yield a major species in the size range M_r 97,000-100,000 when crosslinked to labeled IL-1, suggesting that a protein of M_r 80,000 was responsible for binding IL-1. In contrast, IL-1 receptors characterized in this fashion on human B cells (Matsushima et al., J. Immunol. 136:4496, 1986) displayed an apparent molecular weight of 60,000.

Bron and MacDonald, FEBS Letters 219:365 (1987), disclose immunoprecipitation of murine IL-1 receptor from surface-labeled EL-4 cells using a rabbit polyclonal antiserum directed to IL-1. This work indicated that the murine receptor is a glycoprotein having an apparent molecular weight of approximately 82,000 daltons.

Radiolabeled IL-1 has been used in chemical crosslinking studies and for the detection of receptor in detergent extracts of cells. The results of these experiments, noted above, suggest that a protein of M_r 60,000 or 80,000 is responsible for binding IL-1. The crosslinking of radiolabeled IL-1 to cells has also led to the occasional detection of proteins distinct from the major species of M_r 80,000, suggesting that the IL-1 binding molecule may exist in the membrane as part of a multi-subunit receptor complex.

In order to study the structure and biological characteristics of IL-1 receptors and the role played by IL-1 receptors in the responses of various cell populations to IL-1 stimulation, or to use IL-1 receptors effectively in therapy, diagnosis, or assay, homogeneous compositions of IL-1 receptor are needed. Such compositions are theoretically available via purification of solubilized receptors expressed by cultured cells, or by cloning and expression of genes encoding the receptors. However, prior to the present invention, several obstacles prevented these goals from being achieved.

Even in cell lines known to express detectable levels of IL-1

receptor, the IL-1 receptor is present as a very minor component of total cellular proteins. Moreover, no cell lines were known that expressed high levels of IL-1 receptors constitutively and continuously. For example, the murine EL-4 6.1 cell line expresses
5 detectable levels of IL-1 receptor, but the level of IL-1 receptor expression tends to decay with time, which greatly complicates the process of obtaining sufficient quantities of receptor to provide a useful starting material for purification. Thus, a method of continuously selecting cells for acceptable levels of IL-1 receptor
10 expression, employing fluorescence-activated cell sorting (FACS), was devised.

Additional problems are inherent in attempting to clone mammalian genes encoding IL-1 receptor. Even if a protein composition of sufficient purity can be obtained to permit N-terminal protein
15 sequencing, the degeneracy of the genetic code typically does not permit one to define a suitable probe without considerable additional experimentation. Many iterative attempts may be required to define a probe having the requisite specificity to identify a hybridizing sequence in a cDNA library. To circumvent this problem, a novel
20 direct receptor expression cloning technique was devised to avoid the need for repetitive screening using different probes of unknown specificity. This technique, which has never before been employed, allows direct visualization of receptor expression following transfection of a mammalian cell line with a high expression vector
25 containing a cDNA clone encoding the receptor.

Purified IL-1 receptor compositions will be useful in diagnostic assays for IL-1 or IL-1 receptor, and also in raising antibodies to IL-1 receptor for use in diagnosis or therapy. In addition, purified IL-1 receptor compositions may be used directly in
30 therapy to bind or scavenge IL-1, thereby providing a means for regulating the immune or inflammatory activities of this cytokine.

SUMMARY OF THE INVENTION

The present invention provides DNA sequences consisting
35 essentially of a single open reading frame nucleotide sequence

encoding a mammalian Interleukin-1 receptor (IL-1R) or subunit thereof. Preferably, such DNA sequences are selected from the group consisting of (a) cDNA clones having a nucleotide sequence derived from the coding region of a native IL-1R gene; (b) DNA sequences
5 capable of hybridization to the cDNA clones of (a) under moderately stringent conditions and which encode biologically active IL-1R molecules; and (c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode biologically active IL-1R molecules. The present invention
10 also provides recombinant expression vectors comprising the DNA sequences defined above, recombinant IL-1R molecules produced using the recombinant expression vectors, and processes for producing the recombinant IL-1R molecules utilizing the expression vectors.

The present invention also provides substantially homogeneous
15 protein compositions comprising murine or human IL-1 receptor. The murine molecule is a glycoprotein having a molecular weight of about 82,000 daltons by SDS-PAGE, a binding affinity (K_a) for human IL-1 α of from $3-6 \times 10^9 \text{ M}^{-1}$, and the N-terminal amino acid sequence L E I D V C T E Y P N Q I V L F L S V N E I D I R K.

20 In another aspect, the present invention provides a process for purifying IL-1 receptor, comprising applying a sample comprising IL-1 receptor to an affinity matrix comprising an IL-1 molecule bound to an insoluble support, and eluting bound IL-1 receptor from the affinity matrix. The partially purified IL-1 receptor can be further
25 purified by application to a lectin affinity column and subsequently eluting the IL-1 receptor from the lectin affinity column. The partially purified IL-1 receptor can then be treated by reversed phase high performance liquid chromatography, and eluted as a single peak of absorbance at 280 nanometers which, when analyzed by SDS-PAGE and
30 silver staining, appeared as a single band. As noted above, the native murine IL-1 receptor had an apparent molecular weight of approximately 82,000 daltons as estimated by SDS-PAGE.

The present invention also provides compositions for use in therapy, diagnosis, assay of IL-1 receptor, or in raising antibodies
35 to IL-1 receptors, comprising effective quantities of soluble native

or recombinant receptor proteins prepared according to the foregoing processes. Such soluble recombinant receptor molecules include truncated proteins wherein regions of the receptor molecule not required for IL-1 binding have been deleted. These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a restriction map of cDNA constructs comprising the coding regions of the murine and human IL-1R genes. The murine fragment, isolated from EL-4 6.1 C10 cells and present as an insert in clone GEMBL78, has been deposited with the American Type Culture Collection under deposit accession number ATCC 67563.

Figure 2 is a schematic illustration of the mammalian high expression plasmid pDC201, which is described in greater detail in Example 6.

Figures 3A-3C provide a graphical comparison of the IL-1 binding characteristics of natural and recombinant IL-1 receptors. Figure 3A compares direct binding of ^{125}I -IL-1 α to cells expressing native IL-1 receptor (EL4 6.1 C10) or recombinant receptor (COS-IL-1R); Figure 3B shows the data from Figure 3A replotted in the Scatchard coordinate system. Figure 3C indicates competition for ^{125}I -IL-1 α binding by unlabeled IL-1 α and IL-1 β . In Figure 3, C indicates the concentration of IL-1 added to the binding incubation (molar); r indicates molecules of IL-1 bound per cell.

DETAILED DESCRIPTION OF THE INVENTION

IL-1 α and IL-1 β apparently regulate the metabolism of cells through a common plasma membrane receptor protein. IL-1 receptor from detergent solutions of EL-4 6.1 C10 cells has been stably adsorbed to nitrocellulose with full retention of IL-1 binding activity. This assay system was used to monitor the purification of the IL-1 receptor and to investigate the effects of several chemical modifications on receptor binding activity. IL-1 receptors extracted from EL-4 6.1 C10 cells can be bound to and specifically eluted from IL-1 α coupled to

Sephadex or other suitable affinity chromatography supports.

Purification by the foregoing process resulted in the identification by silver staining of polyacrylamide gels of a protein of M_r 82,000 daltons that was present in fractions exhibiting IL-1 binding activity. Experiments in which the cell surface proteins of EL-4 cells were radiolabeled and ^{125}I labeled receptor was purified by affinity chromatography suggested that the M_r 82,000 protein was expressed on the plasma membrane. N-glycanase treatment of this material showed that 21-35% of the total M_r (82,000) of the receptor was N-linked carbohydrate.

In order to define the chemical properties of the IL-1 receptor, a simple, reproducible and quantitative assay system was devised for the detection of IL-1 receptor in detergent solutions. With this assay, receptor purification can be followed, and changes in receptor binding activity in response to chemical modification of the receptor can be easily monitored.

Binding Assay for IL-1 Receptor

Recombinant human IL-1 β and IL-1 α can be prepared by expression in E. coli and purification to homogeneity as described by Kronheim et al. (Bio/Technology 4:1078, 1986). Recombinant human IL-1 α is preferably expressed as a polypeptide composed of the C-terminal 157 residues of IL-1 α , which corresponds to the M_r 17,500 form of the protein released by activated macrophages. The purified protein is stored at -70°C in phosphate buffered saline as a stock solution of 3 mg/ml. 10 μl (30 μg) aliquots of the stock solution are labeled with sodium (^{125}I) iodide by a modified chloramine-T method described by Dower et al. (Nature 324:266, 1986) and Segal et al. (J. Immunol. 118:1338, 1977). In this procedure, 10 μg rIL-1 α (0.57 nmol) in 10 μl phosphate (0.05 M) buffered saline (0.15 M) pH 7.2 (PBS) are added to 2.5 mCi (1.0 nmol) of sodium iodide in 25 μl of 0.05 M sodium phosphate pH 7.0. The reaction is initiated by addition of 30 μl of 1.4×10^{-4} M chloramine-T (4.2 nmol; Sigma Chemical Co., St. Louis, MO, USA). After 30 minutes on ice the reaction mixture is fractionated by gel filtration on a 1 mL bed volume Biogel P6

(Bio-Rad, Richmond, CA, USA) column. Routinely, 40-50% of ^{125}I is incorporated into protein.

^{125}I -IL-1 α can be purified by gel filtration or other suitable methods and immediately diluted to a working stock solution of 3×10^{-8} M in Roswell Park Memorial Institute (RPMI) 1640 medium comprising 1% (w/v) bovine serum albumin (BSA), 0.1% (w/v) sodium azide, 20 mM Hepes pH 7.4 (binding medium), to avoid radiolysis. Such dilute solutions can be stored for up to one month without detectable loss of receptor binding activity. The specific activity is routinely in the range $1-3 \times 10^{15}$ cpm/mmol (ca 1 atom of iodine per IL-1 α molecule). Typically, the labeled protein is initially (prior to dilution) 100% active as determined by its capacity to elicit IL-2 production from EL-4 6.1 C10 cells. Further, 100% of the ^{125}I cpm can be precipitated by trichloroacetic acid and >95% can be absorbed by IL-1 receptor bearing cells.

EL-4 6.1 C10 cells are propagated in suspension culture as described by MacDonald et al., J. Immunol. 135:3964 (1985). An IL-1 receptor negative variant line of EL-4 cells, EL-4 (M) (ATCC TIB 39), is grown in an identical fashion. Cells are monitored on a weekly basis for IL-1 receptor expression by ^{125}I -IL-1 α binding.

To maintain relatively high levels of receptor expression, cells can be sorted using fluorescence-activated cell sorting (FACS) and fluorescein-conjugated recombinant IL-1 α . Fluorescein-conjugated rIL-1 α (FITC IL-1 α) is prepared by reacting 2.9 nanomoles protein with 100 nanomoles of fluorescein isothiocyanate (Research Organics, Cleveland, Ohio) in a total volume of 70 μl of borate (0.02 M) buffered saline (0.15 M) pH 8.5 for two hours at 37°C. Protein is separated from unconjugated dye by gel filtration on a 1 ml bed volume P6 column, as described by Dower et al. (J. Exp. Med. 162:501, 1985). Using an EPICS C flow cytometer (Coulter Instruments; 488 nm argon laser line, 300 MW, gain 20, PMT voltage 1700), cells providing the highest level fluorescence signal (e.g., the top 1.0% or 0.1%, as desired) are collected and used to establish cell cultures for receptor expression.

For extractions, cells harvested from culture by

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centrifugation are washed once with binding medium and sedimented at 2000 x g for 10 min to form a packed pellet (ca 8×10^8 cells/ml). To the pellet is added an equal volume of PBS containing 1% Triton X-100 and a cocktail of protease inhibitors (2 mM phenylmethylsulphonyl fluoride, 1 μ M pepstatin, 1 μ M leupeptin, and 2 mM O-phenanthroline). The cells are mixed with the extraction buffer by vigorous vortexing and the mixture incubated on ice for 15 minutes; at the end of this time the mixture is centrifuged at 11,000 x g for 30 minutes at 8°C to remove nuclei and other debris. The supernatant is made 0.02% w/v in sodium azide and stored either at 8°C or -70°C, with no loss in IL-1 receptor activity detected for periods of up to six months at either temperature.

For solid phase binding assays, unless otherwise indicated, 1 μ l (4×10^5 cell equivalents) aliquots of extract are placed on dry BA85/21 nitrocellulose membranes (Schleicher & Schuell, Keene, NH) and the membranes kept at room temperature until dry. Dry membranes can be stored at room temperature until use. Under these conditions, receptor binding activity remains stable for up to two months. Prior to use, membranes are reconstituted by incubating for 30 minutes in Tris (0.05 M) buffered saline (0.15 M) pH 7.5 containing 3% w/v BSA to block nonspecific binding sites, washed twice with PBS (20 ml per filter), once with binding medium and cut while wet into 0.9 x 0.9 cm squares with the IL-1 receptor extract at the center. The squares are placed in 24 well trays (Costar, Cambridge, MA) and covered with 200 μ l of binding medium containing 125 I-IL-1 α or 125 I-IL-1 α and unlabeled inhibitors. Trays are then placed on a nutator and incubated in a refrigerator (8°C) for two hours. At the end of this time a 60 μ l aliquot can be taken from each well for determination of unbound 125 I-rIL-1 α . Subsequently, the remaining solution is aspirated and discarded and the nitrocellulose filters washed by adding and aspirating sequentially 1 ml of binding medium and three times 1 ml of PBS to each well. The nitrocellulose squares are then removed and dried on filter paper. Subsequently, they are either placed on Kodak X-omat AR film for twelve hours at -70°C, or placed in 12 x 75 cm glass tubes and counted on a gamma counter.

Brief Description of Tables

Table 1 depicts the cDNA sequence of clone GEMBL78. Nucleotides are numbered from the beginning of the fragment. The CTG codon specifying the leucine residue constituting the N-terminus is underlined at position 282, and the TAG terminator codon which ends the open reading frame is underlined at position 1953.

Tables 2A-2C depict the cDNA sequence and derived amino acid sequence of the coding region of the cDNA shown in Table 1. In Tables 2A-2C, nucleotides and amino acids are numbered from the leucine residue representing the N-terminus of the mature protein. The alternative initiator methionines, N-terminus, and 21 amino acid putative transmembrane region of the murine IL-1 receptor are underlined.

Table 3 depicts a cDNA sequence which includes the complete coding region of the human IL-1R gene. Nucleotides are numbered from the beginning of a fragment, designated R3A, which includes the N-terminus and a short sequence of 5' nontranslated DNA. The CTG codon specifying the leucine residue constituting the N-terminus is underlined at position 135, and the TAG terminator codon which ends the open reading frame is underlined at position 1791.

Tables 4A-4C depict the cDNA sequence and derived amino acid sequence of the coding region of a cDNA encoding human IL-1 receptor. In Tables 4A-4C, nucleotides and amino acids are numbered from the leucine residue (underlined) representing the N-terminus of the mature protein. The 20-amino acid transmembrane region is also underlined.

Table 5 is a comparison of the derived amino acid sequences of the murine and human IL-1 receptors. The transmembrane regions of each protein are underlined, and conserved cysteine residues are indicated by asterisks. Potential N-linked glycosylation sites are indicated by triangles adjacent to asparagine residues.

Definitions

"Interleukin-1 receptor" and "IL-1R" refer to proteins which are capable of binding Interleukin-1 (IL-1) molecules and, in their native configuration as mammalian plasma membrane proteins, presumably

play a role in transducing the signal provided by IL-1 to a cell. As used herein, the term includes analogs of native proteins with IL-1-binding or signal transducing activity. Specifically included are truncated or soluble forms of the IL-1 receptor protein not having
5 a cytoplasmic and transmembrane region. The predicted molecular weight of the murine protein corresponding to the sequence of the mature protein depicted in Tables 2A-2B is 64,597 daltons, while the predicted weight of the precursor is 66,697 daltons. Both of these estimates are exclusive of any glycosylation. The predicted molecular
10 weight of the human protein corresponding to the sequence of the mature protein depicted in Tables 4A-4C is 63,486 daltons, while the predicted weight of the precursor is 65,402 daltons.

"Substantially identical" and "substantially similar," when used to define amino acid sequences, mean that a particular subject
15 sequence, for example, a mutant sequence, varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between reference and subject sequences. For purposes of the present invention, amino acid sequences having greater than 30
20 percent similarity are considered to be substantially similar, and amino acid sequences having greater than 80 percent similarity are considered to be substantially identical. In defining nucleic acid sequences, all subject nucleic acid sequences capable of encoding substantially similar amino acid sequences are considered
25 substantially similar to a reference nucleic acid sequence, and all nucleic acid sequences capable of encoding substantially identical amino acid sequences are considered substantially identical to a reference sequence. For purposes of determining similarity, truncation or internal deletions of the reference sequence should be
30 disregarded. Sequences having lesser degrees of similarity, comparable biological activity, and equivalent expression characteristics are considered to be equivalents. For purposes of the present invention, a "subunit" of an IL-1R is deemed to constitute an amino acid sequence of at least 20 amino acids.

35 "Recombinant," as used herein, means that a protein is

derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a protein essentially free of native
5 endogenous substances and unaccompanied by associated native glycosylation. Protein expressed in most bacterial cultures, e.g., E. coli, will be free of glycan; protein expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

10 "Biologically active," as used throughout the specification as a characteristic of IL-1 receptors, means either that a particular molecule shares sufficient amino acid sequence similarity with the embodiments of the present invention disclosed herein to be capable of binding at least 0.01 nmoles IL-1 per nanomole IL-1 receptor or IL-1
15 receptor analog, or, in the alternative, shares sufficient amino acid sequence similarity to be capable of transmitting an IL-1 stimulus to a cell, for example, as a component of a hybrid receptor construct. Preferably, biologically active IL-1 receptors within the scope of the present invention are capable of binding greater than 0.1 nanomoles
20 IL-1 per nanomole receptor, and most preferably, greater than 0.5 nanomoles IL-1 per nanomole receptor.

"DNA sequence" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure
25 form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading
30 frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with
35 manipulation or expression of the coding regions.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. DNA sequences encoding the proteins provided by this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit.

"Recombinant expression vector" refers to a plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant microbial expression system" means a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as E. coli or yeast such as S. cerevisiae, which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

30

Isolation of cDNAs Encoding IL-1 Receptors

In order to secure the murine coding sequence, a DNA sequence encoding murine IL-1R (mIL-1R) was isolated from a cDNA library prepared by reverse transcription of polyadenylated RNA isolated from the murine cell line EL-4 6.1 C10. The library was screened by direct

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expression of pooled cDNA fragments in monkey COS-7 cells using a mammalian expression vector (pDC201) that uses regulatory sequences derived from SV40 and Adenovirus 2. Transfectants expressing biologically active IL-1R were identified by incubating transfected
5 COS-7 cells with medium containing ^{125}I -IL-1 α , washing the cells to remove unbound labeled IL-1 α , and contacting the cell monolayers with X-ray film to detect concentrations of IL-1 α binding. Transfectants detected in this manner appear as dark foci against a relatively light background.

10 Using this approach, approximately 150,000 cDNAs were screened in pools of approximately 350 cDNAs until assay of one transfectant pool indicated positive foci of IL-1 α binding. A frozen stock of bacteria from this positive pool was grown in culture and plated to provide individual colonies, which were screened until a
15 single clone (clone 78) was identified which directed synthesis of a surface protein with detectable IL-1 binding activity. This clone was isolated, and its insert sequenced to determine the sequence of the murine cDNA set forth in Table 1. The initiator methionine for the full-length translation product of the native murine gene is one of
20 two alternative methionine residues found at positions -19 and -16 of Table 2A. The first amino acid residue of the mature receptor protein was deduced by comparison to an N-terminal amino acid sequence obtained from highly purified preparations of IL-1R derived from EL-4
6.1 C10 cells. This residue is a leucine residue shown at position 1
25 of Table 2A. The 1671 nucleotide coding region corresponding to the mature protein encodes 576 amino acids, including 15 cysteine residues and a 21-amino acid putative transmembrane region. Located N-terminal to the transmembrane region are 7 potential N-glycosylation sites. A cloning vector comprising the full-length murine cDNA, designated
30 GEMBL78, has been deposited with the American Type Culture Collection, Rockville, MD, USA (ATCC) under accession number 67563. The deposit was made under the conditions of the Budapest Treaty.

A probe was constructed from the murine sequence and used to screen human cDNA libraries prepared from cultures of a human T-cell
35 clone grown in the presence of OKT3 antibody and IL-2. cDNA clones

which hybridized to the murine probe were then isolated and sequenced. Using a fragment derived from human cDNA clones, a 1707 nucleotide human coding sequence was obtained and sequenced. The nucleotide sequence of the human cDNA, including 5' and 3' nontranslated sequences, is shown in Table 3. The nucleotide sequence of the human open reading frame and derived amino acid sequence of the human protein is set forth in Tables 4A-4C. This sequence comprises 569 amino acids (including a 17 amino acid signal peptide), including 16 cysteine residues, 13 of which are conserved between the murine and human genes. In addition, the human sequence includes six potential N-glycosylation sites, of which 5 are conserved between murine and human. The amino acid sequence of Tables 4A-4C is numbered from a leucine residue considered to be the likely N-terminus on the basis of comparison to the murine protein. The putative transmembrane region of the human gene is 20 amino acids in length. The sequences of the presumed intracellular portions of the murine and human genes are highly (87%) conserved; the extracellular (78%) and transmembrane regions (63%) are somewhat less conserved, except for the location of cysteines presumably involved in intramolecular disulfide bonding and certain N-glycosylation sites. The derived amino acid sequences of the human and murine genes are compared in Table 5.

The murine and human genes encode integral membrane proteins including intracellular regions having no apparent homology with any known protein sequence and extracellular portions which appear to be organized into domains similar to those of members of the immunoglobulin gene superfamily. Immunoglobulin-like domains typically possess only minimal amino acid similarity but share a common three-dimensional structure consisting of two β -sheets held together by a disulfide bond. The cysteine residues involved in formation of this disulfide bond, as well as a few other critical residues, are highly conserved and occur in the same relative position in almost all members of the family. Members of the immunoglobulin superfamily include not only immunoglobulin constant and variable regions but also a number of other cell surface molecules, many of which are involved in cell-cell interactions.

Like most mammalian genes, mammalian IL-1Rs are presumably encoded by multi-exon genes. Alternative mRNA constructs which can be attributed to different mRNA splicing events following transcription, and which share large regions of identity or similarity with the cDNAs
5 claimed herein, are considered to be within the scope of the present invention.

In its nucleic acid embodiments, the present invention provides DNA sequences encoding mammalian IL-1Rs. Examples of mammalian IL-1Rs include primate IL-1R, human IL-1R, murine, canine,
10 feline, bovine, ovine, equine and porcine IL-1Rs. IL-1R DNAs are preferably provided in a form which is capable of being expressed in a recombinant transcriptional unit under the control of mammalian, microbial, or viral transcriptional or translational control elements. For example, a sequence to be expressed in a microorganism will
15 contain no introns. In preferred aspects, the DNA sequences comprise at least one, but optionally more than one sequence component derived from a cDNA sequence or copy thereof. Such sequences may be linked or flanked by DNA sequences prepared by assembly of synthetic oligo-nucleotides. However, synthetic genes assembled exclusively from
20 oligonucleotides could be constructed using the sequence information provided herein. Exemplary sequences include those substantially identical to the nucleotide sequences depicted in Tables 2A-2C. Alternatively, the coding sequences may include codons encoding one or more additional amino acids located at the N-terminus, for example, an
25 N-terminal ATG codon specifying methionine linked in reading frame with the nucleotide sequence. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence; exemplary DNA embodiments are those corresponding to the sequence of nucleotides 1-1671 of Tables 2A-2C, and nucleotides
30 1-1656 of Tables 4A-4C. Other embodiments include sequences capable of hybridizing to the sequence of Tables 2A-2C or 4A-4C under moderately stringent conditions (50°C, 2 x SSC) and other sequences degenerate to those described above which encode biologically active IL-1R polypeptides.

35 The present invention also provides expression vectors for

producing useful quantities of purified IL-1R. The vectors can comprise synthetic or cDNA-derived DNA fragments encoding mammalian IL-1Rs or bioequivalent homologues operably linked to regulatory elements derived from mammalian, bacterial, yeast, bacteriophage, or viral genes. Useful regulatory elements are described in greater detail below. Following transformation, transfection or infection of appropriate cell lines, such vectors can be induced to express recombinant protein.

Mammalian IL-1Rs can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems could also be employed to produce mammalian IL-1R using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can be employed to express recombinant protein. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (Cell 23:175, 1981), and other cell lines capable of expressing an appropriate vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and termination sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. Additional details regarding the use of a mammalian high expression vector to produce a recombinant mammalian IL-1R are provided in Examples 4 and 6, below. Exemplary vectors can be constructed as disclosed by Okayama and Berg (Mol. Cell. Biol. 3:280,

1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (Molecular Immunol. 23:935, 1986).

Yeast systems, preferably employing Saccharomyces species such as S. cerevisiae, can also be employed for expression of the recombinant proteins of this invention. Yeast of other genera, for example, Pichia or Kluyveromyces, have also been employed as production strains for recombinant proteins.

Generally, useful yeast vectors will include origins of replication and selectable markers permitting transformation of both yeast and E. coli, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed yeast gene to induce transcription of a downstream structural sequence. Such promoters can be derived from yeast transcriptional units encoding highly expressed genes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate reading frame with translation initiation and termination sequences, and, preferably, a leader sequence capable of directing secretion of translated protein into the extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide (e.g., Asp-Tyr-Lys- (Asp)₄-Lys) or other sequence imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in E. coli (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible alcohol dehydrogenase 2 (ADH2) promoter. The ADH2 promoter has been described by Russell et al. (J. Biol. Chem. 258:2674, 1982), and Beier et al. (Nature 300:724, 1982). Such vectors may also include a yeast TRP1 gene as a selectable marker and the yeast 2 μ origin of replication. A yeast leader sequence, for

example, the α -factor leader which directs secretion of heterologous proteins from a yeast host, can be inserted between the promoter and the structural gene to be expressed (see Kurjan et al., U.S. Patent 4,546,082; Kurjan et al., Cell 30:933 (1982); and Bitter et al., Proc. Natl. Acad. Sci. USA 81:5330, 1984). The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those skilled in the art; an exemplary technique is described by Hinnen et al. (Proc. Natl. Acad. Sci. USA 75:1929, 1978), selecting for Trp⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μ g/ml adenine and 20 μ g/ml uracil.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μ g/ml adenine and 80 μ g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Useful expression vectors for bacterial use are constructed by inserting a DNA sequence encoding mammalian IL-1R together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium, and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

Expression vectors are conveniently constructed by cleavage of cDNA clones at sites close to the codon encoding the N-terminal residue of the mature protein. Synthetic oligonucleotides can then be used to "add back" any deleted sections of the coding region and to provide a linking sequence for ligation of the coding fragment in appropriate reading frame in the expression vector, and optionally a

codon specifying an initiator methionine.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857 thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in E. coli strain JMB9 (ATCC 37092) and pPLc28, resident in E. coli RR1 (ATCC 53082). Other useful promoters for expression in E. coli include the T7 RNA polymerase promoter described by Studier et al. (J. Mol. Biol. 189: 113, 1986), the lacZ promoter described by Lauer (J. Mol. Appl. Genet. 1:139-147, 1981) and available as ATCC 37121, and the tac promoter described by Maniatis (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, p 412) and available as ATCC 37138.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Cells are grown, for example, in a 10 liter fermenter employing conditions of maximum aeration and vigorous agitation. An antifoaming agent (Antifoam A) is preferably employed. Cultures are grown at 30°C in the superinduction medium disclosed by Mott et al. (Proc. Natl. Acad. Sci. USA 82:88, 1985), alternatively including antibiotics, derepressed at a cell density corresponding to $A_{600} = 0.4-0.5$ by elevating the temperature to 42°C, and harvested

from 2-20, preferably 3-6, hours after the upward temperature shift. The cell mass is initially concentrated by filtration or other means, then centrifuged at 10,000 x g for 10 minutes at 4°C followed by rapidly freezing the cell pellet.

5 Preferably, purified mammalian IL-1Rs or bioequivalent homologues are prepared by culturing suitable host/vector systems to express the recombinant translation products of the synthetic genes of the present invention, which are then purified from culture media.

 An alternative process for producing purified IL-1R involves
10 purification from cell culture supernatants or extracts. In this approach, a cell line which elaborates useful quantities of the protein is employed. Supernatants from such cell lines can be optionally concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon
15 ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix as previously described. For example, a suitable affinity matrix can comprise an IL-1 or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for
20 example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising
25 sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred.

 Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can
30 be employed to further purify an IL-1R composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

 Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or
35 more concentration, salting-out, aqueous ion exchange or size

exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant mammalian IL-1R can be disrupted by any convenient method, including freeze-thaw
5 cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express mammalian IL-1R as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be
10 purified by methods analogous to those disclosed by Urdal et al. (J. Chromatog. 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

In its various embodiments, the present invention provides
15 substantially homogeneous recombinant mammalian IL-1R polypeptides free of contaminating endogenous materials, with or without associated native-pattern glycosylation. The native murine IL-1R molecule is recovered from cell culture extracts as a glycoprotein having an apparent molecular weight by SDS-PAGE of about 82 kilodaltons (kD).
20 IL-1Rs expressed in mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern to the native molecules, depending upon the expression system. Expression of IL-1R DNAs in bacteria such as E. coli provides non-glycosylated molecules having an apparent molecular
25 weight of about 60 kD by SDS-PAGE under nonreducing conditions.

Recombinant IL-1R proteins within the scope of the present invention also include N-terminal methionyl murine and human IL-1Rs. Additional embodiments include soluble truncated versions wherein certain regions, for example, the transmembrane region and
30 intracellular domains, are deleted, providing a molecule having an IL-1-binding domain only. Also contemplated are mammalian IL-1Rs expressed as fusion proteins with a polypeptide leader comprising the sequence Asp-Tyr-Lys-(Asp₄)-Lys, or with other suitable peptide or protein sequences employed as aids to expression in microorganisms or
35 purification of microbially-expressed proteins.

Bioequivalent homologues of the proteins of this invention include various analogs, for example, truncated versions of IL-1Rs wherein terminal or internal residues or sequences not needed for biological activity are deleted. Other analogs contemplated herein are those in which one or more cysteine residues have been deleted or replaced with other amino acids, for example, neutral amino acids. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present, or modification of the protein sequence to eliminate one or more N-linked glycosylation sites.

As used herein, "mutant amino acid sequence" refers to a polypeptide encoded by a nucleotide sequence intentionally made variant from a native sequence. "Mutant protein" or "analog" means a protein comprising a mutant amino acid sequence. "Native sequence" refers to an amino acid or nucleic acid sequence which is identical to a wild-type or native form of a gene or protein. The terms "KEX2 protease recognition site" and "N-glycosylation site" are defined below. The term "inactivate," as used in defining particular aspects of the present invention, means to alter a selected KEX2 protease recognition site to retard or prevent cleavage by the KEX2 protease of Saccharomyces cerevisiae, or to alter an N-glycosylation site to preclude covalent bonding of oligosaccharide moieties to particular amino acid residues by the cell.

Site-specific mutagenesis procedures can be employed to inactivate KEX2 protease processing sites by deleting, adding, or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites. The resulting analogs are less susceptible to cleavage by the KEX2 protease at locations other than the yeast α -factor leader sequence, where cleavage upon secretion is intended.

Many secreted proteins acquire covalently attached

carbohydrate units following translation, frequently in the form of oligosaccharide units linked to asparagine side chains by N-glycosidic bonds. Both the structure and number of oligosaccharide units attached to a particular secreted protein can be highly variable, resulting in a wide range of apparent molecular masses attributable to a single glycoprotein. mIL-1R is a glycoprotein of this type. Attempts to express glycoproteins in recombinant systems can be complicated by the heterogeneity attributable to this variable carbohydrate component. For example, purified mixtures of recombinant glycoproteins such as human or murine granulocyte-macrophage colony stimulating factor (GM-CSF) can consist of from 0 to 50% carbohydrate by weight. Miyajima et al. (EMBO Journal 5:1193, 1986) reported expression of a recombinant murine GM-CSF in which N-glycosylation sites had been mutated to preclude glycosylation and reduce heterogeneity of the yeast-expressed product.

The presence of variable quantities of associated carbohydrate in recombinant glycoproteins complicates purification procedures, thereby reducing yield. In addition, should the glycoprotein be employed as a therapeutic agent, a possibility exists that recipients will develop immune reactions to the yeast carbohydrate moieties, requiring therapy to be discontinued. For these reasons, biologically active, homogeneous analogs of immunoregulatory glycoproteins having reduced carbohydrate may be desirable for therapeutic use.

Functional mutant analogs of mammalian IL-1Rs having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques as described below. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A¹-Z, where A¹ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or

inserting a non-Z amino acid between A¹ and Z, or an amino acid other than Asn between Asn and A¹. Preferably, substitutions are made conservatively; i.e., the most preferred substitute amino acids are those having physicochemical characteristics resembling those of the
5 residue to be replaced. Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion upon biological activity should be considered.

In addition to the particular analogs described above, numerous DNA constructions including all or part of the nucleotide
10 sequences depicted in Tables 2A-2C or 4A-4C, in conjunction with oligonucleotide cassettes comprising additional useful restriction sites, can be prepared as a matter of convenience. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling
15 ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene
20 having particular codons altered according to the substitution, deletion, or insertion required. By way of example, Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (Biotechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U. S.
25 Patent No. 4,518,584 disclose suitable techniques, and are incorporated by reference herein.

In one embodiment of the present invention, the amino acid sequence of IL-1R is linked to a yeast α -factor leader sequence via an N-terminal fusion construct comprising a nucleotide encoding the
30 peptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK). The latter sequence is highly antigenic and provides an epitope reversibly bound by specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue
35 immediately following the Asp-Lys pairing. Fusion proteins capped

with this peptide may also be resistant to intracellular degradation in *E. coli*. An alternative construction is Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-Glu-Ile-Gly-Arg, which provides a Factor X recognition site immediately downstream from the enterokinase site.

5 The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

10 Example 1

Preparation of IL-1 α Affinity Matrix and Affinity Purification of Receptor from Surface Labeled EL-4 6.1 C10 Cells

Cell surface proteins on EL-4 6.1 C10 cells were radiolabeled with ^{125}I by the glucose oxidase-lactoperoxidase method disclosed by Cosman et al. (Molecular Immunol. 23:935, 1986). Labeled
15 cells were pelleted by centrifugation, washed three times with PBS, and extracted with PBS containing 1% Triton X-100 and the cocktail of protease inhibitors described in the assay protocol detailed above. The Triton X-100 extract was spun for 10 minutes in an Eppendorf
20 microcentrifuge and the supernatant was stored at -70°C .

Recombinant IL-1 α was coupled to cyanogen bromide activated Sepharose 4B (Pharmacia, Piscataway, NJ, USA) or to Affigel-10 (Bio-Rad, Richmond, CA, USA) according to the manufacturer's suggestions. For example, to a solution of IL-1 α (1.64 mg/ml in 9.5
25 ml PBS), 3 ml were added of swollen, acid-washed, CNBR-activated Sepharose. The solution was rocked overnight at 4°C and an aliquot of the supernatant was tested for protein by a fluorescamine protein assay as described by Udenfriend et al. (Science 178:871, 1972), using BSA as a standard. Ninety-eight percent of the protein had coupled to
30 the gel, suggesting that the column had a final load of 5.1 mg IL-1 α per ml gel. Three hundred μl of 1 M glycine-ethyl-ester (Sigma Chemical Co., St. Louis, MO, USA) were added to the slurry to block any unreacted sites on the gel.

The gel was washed extensively with 0.1 M glycine buffer pH
35 3.0 containing 0.1% Triton X-100, PBS containing 0.1% Triton X-100,

RIPA buffer (0.05 M Tris-HCl pH 7.5, 0.15 M NaCl, 1% NP40, 1% sodium deoxycholate, 0.1% SDS), and PBS containing 0.1% Triton X-100 and 10 mM ATP. Small columns (200 μ l) were prepared in disposable polypropylene holders (Bio-Rad, Richmond, CA, USA) and washed with PBS
5 containing 1% Triton X-100. Aliquots of 100 μ l of 125 I-labeled extract were applied to a column, which was then washed with PBS containing 1% Triton X-100, RIPA buffer, PBS containing 0.1% Triton X-100 and 10 mM ATP, and PBS with 1% Triton X-100.

The IL-1 receptor on murine T cells is a robust structure
10 capable of binding 125 I-IL-1 α in Triton X-100 detergent solutions. To be able to recover receptor from such an affinity matrix, a mild elution procedure is necessary. Mild acid treatment can cause rapid dissociation of preformed IL-1 α /IL-1 receptor complexes. Based upon this observation, pH 3.0 glycine HCl buffer containing 0.1% Triton
15 X-100 were used to elute receptor from the IL-1 α affinity columns, which was collected in 0.05 ml fractions. The presence of receptor in the fractions was detected by dot blot as described above, using 125 I-labeled IL-1 α .

Analysis by SDS-PAGE proceeded as follows. To 50 μ l of each
20 column fraction was added 50 μ l of 2 x SDS sample buffer (0.125 M Tris HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). The solution was placed in a boiling water bath for three minutes and aliquots of 40 μ l were applied to the sample well of a 10% polyacrylamide gel which was set up and run according to the method of
25 Laemmli (Nature 227:680, 1970). Gels were fixed and stained using 0.25% Coomassie brilliant blue in 25% isopropanol, 10% acetic acid), destained in 25% isopropanol, 10% acetic acid, treated with Enhance (New England Nuclear, Boston, MA, USA), dried and exposed to Kodak X-omat AR film at -70°C. Molecular weight markers, labeled with 14 C,
30 were obtained from New England Nuclear, and included: cytochrome C (M_r 12,300), lactoglobulin A (M_r 18,367), carbonic anhydrase (M_r 31,000), ovalbumin (M_r 46,000), bovine serum albumin (M_r 69,000), phosphorylase B (M_r 97,400) and myosin (M_r 200,000). Alternatively, fractions having receptor activity were analyzed by SDS polyacrylamide gel
35 electrophoresis followed by silver staining as previously described by

Urdal et al. (Proc. Natl. Acad. Sci. USA 81:6481, 1984).

Dot blot analysis of fractions eluted from the IL-1 α affinity matrix showed that IL-1 binding activity was detected in fractions that were collected after pH 3.0 glycine buffer was applied to the column. Fractions that scored positive in this assay, when analyzed by SDS-PAGE, revealed that a protein of M_r 82,000 could be detected upon developing the gel with silver stain. To determine which of the proteins detected by silver stain were expressed on the cell surface, EL-4 6.1 cells were surface labeled with ^{125}I by the lactoperoxidase-glucose oxidase procedure. Radiolabeled cells were then extracted with PBS containing 1% Triton X-100 and aliquots of the detergent extract applied to an IL-1 α affinity matrix. Fractions that were collected from this column, following application to the column of pH 3.0 glycine buffer, contained a radiolabeled protein of M_r 82,000.

15

Example 2

Comparison of Properties of Cellular IL-1 Receptor and IL-1 Receptor Isolated from Cell Extracts

In a preliminary experiment, the binding properties of the IL-1 receptor were compared in intact EL-4 6.1 C10 cells and after extraction from cells. 3.8×10^6 EL-4 6.1 C10 cells were divided into two equal aliquots, one of which was extracted as described above. The remaining cells were resuspended at 3.8×10^7 cells/ml and used for direct binding studies. Extract was adsorbed to nitrocellulose and used for solid phase binding studies employing various concentrations of ^{125}I -IL-1 α with or without unlabeled IL-1. After washing and drying, the nitrocellulose filters were first counted for bound ^{125}I -IL-1 α and subsequently placed on film for autoradiography. Nonspecific background was measured in the presence of 5.7×10^{-7} M unlabeled rIL-1 β . The data obtained showed that ^{125}I -IL-1 α was bound to the extract on nitrocellulose in an IL-1 concentration-dependent fashion, and that the ^{125}I -IL-1 α was specifically bound to the region of the blot where extract is present. Further, binding could be extensively blocked by inclusion of unlabeled IL-1 α in the incubation

35

mixture.

The comparison further indicated that not only were the levels of receptor the same in both instances, but that the receptors after adsorption to nitrocellulose exhibited an affinity for ligand which was indistinguishable from that of the receptor in intact cells. No significant difference between the number of receptors detected on intact cells and those detected following detergent extraction was found. This is consistent with the view that the majority of the receptors were present on the external face of the plasma membrane in intact cells.

To measure the specificity of binding of IL-1 receptors on nitrocellulose filters, two μ l of EL-4 6.1 C10 extract were applied to nitrocellulose filters, dried, blocked and assayed as described above. The following proteins were tested for their capacity to inhibit ^{125}I -IL-1 α binding: human rIL-1 α (7.62×10^{-7} M), human rIL-1 β (7.62×10^{-7} M), human IL-2 (8.9×10^{-7} M), murine IL-3 (7.5×10^{-4} M), murine-GM-CSF (7.5×10^{-7} M), recombinant murine IL-4 (5×10^{-9} M), human epidermal growth factor 3 $\mu\text{g/ml}$, fibroblast growth factor 1 $\mu\text{g/ml}$, rat submandibular gland nerve growth factor (2 $\mu\text{g/ml}$), bovine insulin (1×10^{-7} M), human luteinizing hormone (1 $\mu\text{g/ml}$), human growth hormone (1.7×10^{-7} M), thyroid stimulating hormone (1 $\mu\text{g/ml}$), and follicle stimulating hormone (1 $\mu\text{g/ml}$). All incubations were done with 1.9×10^{-10} M ^{125}I -IL-1 α .

This experiment demonstrated that extracted receptor retains the same specificity as that previously demonstrated for intact cells. As found with intact cells, only IL-1 α and IL-1 β produced any significant inhibition of ^{125}I -IL-1 α binding. The data showed that unlabeled IL-1 α and IL-1 β produced >90% inhibition of ^{125}I -IL-1 α binding, while no significant blockade was observed with any of the other hormones.

To determine whether receptor in detergent solution would bind IL-1 with an affinity equal to that of receptor in cell membranes, or adsorbed to nitrocellulose, a third experiment was performed in which the nitrocellulose dot blot binding assay was used to test the capacity of an EL-4 6.1 C10 extract in Triton X-100

solution to inhibit binding of ^{125}I -IL-1 α to the solid phase. EL-4 6.1 C10 extracts were adsorbed to nitrocellulose, dried, blocked and incubated with mixture of ^{125}I -IL-1 α and extracts containing receptors in detergent solution.

5 The concentration of receptor in the solution phase was estimated from a saturation binding curve to 1 μl aliquots blotted on nitrocellulose, allowing receptors/ μl to be calculated and hence IL-1 receptor concentration (M). The extract was diluted through PBS Triton X-100 solution (0.5% Triton) to keep the detergent
10 concentration constant. The inhibition curve showed that in solution, the receptor bound to ^{125}I -IL-1 α with a K_a ($4.5 \pm 0.5 \times 10^9 \text{ M}^{-1}$) that is the same as that of receptor on the solid phase or in membranes. Further, the close fit between the theoretical curve, which is based on a simple competitive inhibition model, and the data was consistent
15 with the hypothesis that a single type of IL-1 binding protein was present in the membrane extract.

 In order to examine the integrity of the receptor as a function of the concentration of total EL-4 6.1 C10 membrane proteins, a fourth experiment was done. Mixtures of EL-4 6.1 C10 extract in
20 various proportions ranging from 10 to 100% were made either with an extract from cells not expressing the IL-1 receptor, EL-4 (M) cells, or with PBS Triton X-100 (0.5%). Each mixture was analyzed for receptor concentration, and affinity of ^{125}I -IL-1 α binding by quantitative dot blot binding. Receptor concentration decreased
25 linearly with the percentage of EL-4 6.1 C10 extract present, whether membrane protein concentration was maintained at a constant level or not. In both series of mixtures the affinity of the receptor for ^{125}I -IL-1 α remained constant. These data are consistent with one of two hypotheses, either the receptor binding function is contained
30 within a single polypeptide chain or, if the functional receptor requires two or more subunits for IL-1 binding, these are sufficiently tightly associated that dilution through detergent does not separate them.

Example 3

Purification of IL-1 Receptor to Homogeneity and
Determination of N-terminal Sequence

300-500 liters of EL-4 6.1 Cl0 cells were grown to saturation
5 under the conditions previously described, harvested, and extracted
with PBS-1% Triton X-100. The detergent extract was applied to an
IL-1 α affinity column and the column washed as previously described.
Fractions containing IL-1 receptor were detected by the 125 I-IL-1 α dot
10 blot procedure following elution of the column with 0.1 M glycine HCl
pH 3.0 containing 0.1% Triton X-100. Aliquots of the fractions were
analyzed by SDS polyacrylamide gel electrophoresis.

This partially purified IL-1 receptor composition prepared by
affinity chromatography on Affigel-IL-1 α was adjusted to contain the
following buffer composition: 10 mM Tris-HCl, pH 8, 250 mM NaCl, 0.5
15 mM MgCl₂, 0.5 mM MnCl₂, 0.5 mM CaCl₂, and 0.01 % (v/v) Triton X-100
(WGA buffer). The IL-1 receptor composition was then applied to a 1
ml column of wheat germ agglutinin (WGA) bound to Sepharose CL-6B,
equilibrated with WGA buffer. Following application of the IL-1
receptor composition, the WGA column was washed with 20 ml of WGA
20 buffer followed by 10 mM Tris HCl, pH 8, 0.01% (v/v) Triton X-100.
The IL-1 receptor protein was eluted from the WGA column with 10 mM
Tris-HCl, pH 8, 0.5 M N-acetylglucosamine, and 0.01% (v/v) Triton
X-100. The presence of biologically active IL-1 receptor was detected
by the 125 I-IL-1 α dot blot procedure. The fractions were also
25 analyzed by SDS polyacrylamide gel electrophoresis followed by silver
staining.

Material eluting from the WGA column was applied to a C8
RP-HPLC column. The C8 RP-HPLC column (Brownlee Labs RP-300, 1 mm X
50 mm) was previously equilibrated with 0.1% (v/v) trifluoroacetic
30 acid (TFA) in HPLC grade H₂O, at a flow rate of 50 μ l/min. Following
application of the IL-1 receptor containing material, the C 8 RP-HPLC
column was washed with 0.1% (v/v) TFA in H₂O at 50 μ l/min until the
absorbance at 280 nm returned to baseline. The IL-1 receptor protein
was eluted from the column by running a linear gradient of 0.1% (v/v)
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TFA in acetonitrile from 0-100% at a rate of 1% per minute. Aliquots of the fractions were analyzed by SDS polyacrylamide gel electrophoresis. The IL-1 receptor protein was found to consist of a single band on an SDS polyacrylamide gel migrating with a molecular weight of 82,000.

The purified IL-1 receptor protein was analyzed by Edman degradation using an Applied Biosystems Model 470A protein sequencer. The protein (150 picomoles) was not modified before analysis. The results of the N-terminal protein sequence analysis of the IL-1 receptor indicated the following sequence of amino acid residues: NH₂-Leu-Glu-Ile-Asp-Val-Cys-Thr-Glu-Tyr-Pro-Asn-Gln-Ile-Val-Leu-Phe-Leu-Ser-Val-Asn-Glu-Ile-Asp-Ile-Arg-Lys.

This protein sequence was found to be unique when compared to the March 17, 1987 release of the Protein Sequence Database of the Protein Identification Resource of the National Biomedical Research Foundation. This release of the database contained 4,253 sequences consisting of 1,029,056 residues.

Example 4

Isolation of cDNA Encoding Murine IL-1R by Direct Expression of Active Protein in COS-7 Cells

A cDNA library was constructed by reverse transcription of polyadenylated mRNA isolated from total RNA extracted from EL-4 6.1 C10 cells by a procedure similar to that of Chirgwin et al. (Biochem. 18:5294, 1979). Briefly, the cells were lysed in a guanidinium isothiocyanate solution, and the lysate layered over a pad of CsCl and centrifuged until the RNA had pelleted. The RNA pellet was resuspended and further purified by protease digestion, organic extraction and alcohol precipitation. Poly A⁺ RNA was isolated by oligo dT cellulose chromatography and double-stranded cDNA was prepared by a method similar to that of Gubler and Hoffman (Gene 25:263, 1983). Briefly, the RNA was copied into cDNA by reverse transcriptase using either oligo dT or random oligonucleotides as primer. The cDNA was made double-stranded by incubation with E. coli DNA polymerase I and RNase H, and the ends made flush by further

incubation with T₄ DNA polymerase. The blunt-ended cDNA was ligated into SmaI-cut dephosphorylated pDC201 vector DNA.

The eukaryotic high expression vector pDC201 was assembled from SV40, adenovirus 2, and pBR322 DNA comprising, in sequence: (1) an SV40 fragment containing the origin of replication, early and late promoters, and enhancer; (2) an adenovirus 2 fragment containing the major late promoter, the first exon and part of the first intron of the tripartite late leader; (3) a synthetic sequence comprising a HindIII site, a splice acceptor site, the second and third exons of the adenovirus 2 tripartite leader and a multiple cloning site including a SmaI site; (4) additional SV40 sequences containing early and late polyadenylation sites; (5) adenovirus 2 sequences including the virus-associated RNA genes; and (6) pBR322 elements for replication in E. coli.

The resulting EL-4 6.1 C10 cDNA library in pDC201 was used to transform E. coli strain DH5 α , and recombinants were plated to provide approximately 350 colonies per plate and sufficient plates to provide approximately 25,000 total colonies per screen. Colonies were scraped from each plate, pooled, and plasmid DNA prepared from each pool. The pooled DNA was then used to transfect a sub-confluent layer of monkey COS-7 cells using DEAE-dextran followed by chloroquine treatment, as described by Luthman et al. (Nucleic Acids Res. 11:1295, 1983) and McCutchan et al. (J. Natl. Cancer Inst. 41:351, 1986). The cells were then grown in culture for three days to permit transient expression of the inserted sequences. After three days, cell culture supernatants were discarded and the cell monolayers in each plate assayed for IL-1 binding as follows. Three ml of RPMI medium containing 3×10^{-10} M ¹²⁵I-IL-1 α was added to each plate and the plates incubated for 2 hours at 8°C. This medium was then discarded, and each plate was washed with 10 ml RPMI 1640 medium [containing no labeled IL-1 α]. The edges of each plate were then broken off, leaving a flat disk which was contacted with X-ray film for 72 hours at -70°C using an intensifying screen. IL-1 binding activity was visualized on the exposed films as a dark focus against a relatively uniform background.

After approximately 150,000 recombinants from the library had

been screened in this manner, one transfectant pool was observed to provide IL-1 binding foci which were clearly apparent against the background exposure.

A frozen stock of bacteria from the positive pool was then used to obtain plates of approximately 350 colonies. Replicas of these plates were made on nitrocellulose filters, and the plates were then scraped and plasmid DNA prepared and transfected as described above to identify a positive plate. Bacteria from individual colonies from the nitrocellulose replicas of this plate were grown in 2 ml cultures, which were used to obtain plasmid DNA, which was transfected into COS-7 cells as described above. In this manner, a single clone, clone 78, was isolated which was capable of inducing expression of IL-1R in COS cells. The insert was subcloned into a plasmid derived from pBR322 (GEMBL) and sequenced by conventional techniques. The sequence is set forth in Table 1.

Example 5

Isolation of Human cDNA Clones Which Hybridize to Murine IL-1 Receptor Probe DNAs

A cDNA polynucleotide probe was prepared from the 2356 base pair (bp) fragment of clone 78 (see Example 4) by nick-translation using DNA polymerase I. The method employed was substantially similar to that disclosed by Maniatis et al. (*supra*, p. 109).

A cDNA library was constructed by reverse transcription of polyadenylated mRNA isolated from total RNA extracted from the cultured cells of a human T-cell line designated clone 22, described by Acres et al. (*J. Immunol.* 138:2132, 1987). These cells were cultured in RPMI 1640 medium plus 10% fetal bovine serum as described by Acres et al. (*supra*), in the presence of 10 ng/ml OKT3 antibody and 10 ng/ml human IL-2. The cDNA was rendered double-stranded using DNA polymerase I, blunt-ended with T4 DNA polymerase, methylated with EcoRI methylase to protect EcoRI cleavage sites within the cDNA, and ligated to EcoRI linkers. The resulting constructs were digested with EcoRI to remove all but one copy of the linkers at each end of the cDNA, and ligated to EcoRI-cut and dephosphorylated arms of

bacteriophage λ gt10 (Huynh et al., DNA Cloning: A Practical Approach, Glover, ed., IRL Press, pp. 49-78). The ligated DNA was packaged into phage particles using a commercially available kit (Stratagene Cloning Systems, San Diego, CA, USA 92121) to generate a library of

- 5 recombinants. Recombinants were plated on E. coli strain C600(hf1-) and screened by standard plaque hybridization techniques under conditions of moderate stringency (50°C, 6 x SSC).

- Following several rounds of screening, nine clones were isolated from the library which hybridized to the cDNA probe. The
10 clones were plaque purified and used to prepare bacteriophage DNA which was digested with EcoRI. The digests were electrophoresed on an agarose gel, blotted onto nylon filters, and retested for hybridization. The clones were digested with EcoRI followed by preparative agarose gel electrophoresis, then subcloned into an
15 EcoRI-cut derivative (pGEMBL) of the standard cloning vector pBR322 containing a polylinker having a unique EcoRI site, a BamHI site and numerous other unique restriction sites. An exemplary vector of this type is described by Dente et al. (Nucleic Acids Research 11:1645, 1983).

- 20 Restriction mapping and sequencing of a 4.8 kb human IL-1R clone indicated that the clone included a sequence encoding 518 amino acids which exhibited 80% amino acid sequence identity to the corresponding murine sequence in the extracellular, or N-terminal region distal to the transmembrane region, 63% identity in the
25 transmembrane region, and 87% identity in the cytoplasmic, or C-terminal region. In addition, several cysteine residues and most N-linked glycosylation sites between the mouse and human sequences were conserved. A 440 bp EcoRI-NsiI fragment derived from the 5' portion of the human IL-1R clone was ^{32}P -labeled by nick-translation
30 as described above and used to screen a cDNA library produced by randomly-priming clone 22 mRNA prepared as described above. 23 clones which hybridized to the probe were isolated and analyzed by restriction mapping. Sequencing of one of these clones provided the sequence information corresponding to the remaining N-terminal 34
35 amino acids of the human protein. The coding and deduced amino acid

sequence of the complete coding region of human IL-1R is shown in Tables 4A-4C.

Example 6

5 Expression of Recombinant IL-1 Receptor Using a High-Efficiency Mammalian Expression System

The mammalian expression plasmid pDC201, depicted in Figure 2, is designed to express cDNA sequences inserted at its multiple cloning site (MCS) when transfected into mammalian cells. Referring
10 now to Figure 2, pDC201 includes the following components: SV40 (hatched box) contains SV40 sequences from coordinates 5171-270 including the origin of replication, enhancer sequences and early and late promoters. The fragment is oriented so that the direction of transcription from the early promoter is as shown by the arrow.
15 Ad-MLP (open box) contains adenovirus-2 sequences from coordinates 5779-6231 including the major late promoter, the first exon and part of the intron between the first and second exons of the tripartite leader. TPL (stippled box) contains a synthetic DNA sequence specifying adenovirus-2 sequences 7056-7172, 9634-9693 (containing the
20 acceptor splice site of the second exon of the tripartite leader, the second exon and part of the third exon of the tripartite leader) and a multiple cloning site (MCS) containing sites for KpnI, SmaI, and BglIII. pA (hatched box) contains SV40 sequences from 4127-4100 and 2770-2533 that include the polyadenylation and termination signals for
25 early transcription. VA (solid box) contains adenovirus-2 sequences from 10226-11555 that include the virus-associated RNA genes (VAI and VAII). The solid lines are derived from pBR322 and represent (starting after the pA sequences and proceeding clockwise) coordinates 29-23, 651-185 (at which point the VA sequences are inserted), 29-1,
30 4363-2486, and 1094-375. pDC201 is a derivative of pMLSV, previously described by Cosman et al., Molec. Immunol. 23:935 (1986).

To express recombinant IL-1 receptor, COS cells were grown and transfected as described by Cosman et al., supra, with the plasmid DNA from a 1.5 ml culture of E. coli transformed with pDC201
35 having an IL-1R cDNA insert (clone 78). After 72 hours of culture

cells were harvested by washing once with 10 ml of PBS and then treating for 20 minutes at 37°C with an EDTA solution (sodium phosphate 0.05 M, sodium chloride 0.15 M, EDTA 0.005 M, pH 7.4) followed by scraping. For comparisons, COS cells were transfected
5 with a pDC201 control vector containing no insert, and EL-4 6.1 C10 cells and EL-4 M cells (an IL-1 receptor-negative variant of EL-4 cells) were grown and harvested as described by McDonald et al., J. Immunol. 135:3964 (1985).

At saturating DNA concentrations, the transfected COS cell
10 monolayer contained an average of 45,000 sites per cell. Since the parental COS cells expressed only about 500 receptors per cell, it can be calculated that more than 98% of all IL-1 receptors in the transfected population were recombinant. Flow cytometry using FITC-IL-1 α revealed that only 4.2% of the cells stained brightly;
15 therefore, each of these transfected COS cells contained about 1.1×10^6 IL-1 binding sites.

The plasma membrane proteins of EL-4 6.1 C10 cells and of COS cells transfected with vector DNA containing cDNA encoding the IL-1 receptor (clone 78) were labeled with ^{125}I as described in Example 1,
20 above. Cells were subsequently extracted with PBS containing 1% Triton X-100 and a cocktail of protease inhibitors (2 mM phenylmethyl sulphonyl fluoride, 1 mM pepstatin, 1 mM leupeptin, and 2 mM O-phenanthroline). Detergent extracts were subjected to affinity chromatography as described in Example 1 on Affigel-10 (Biorad,
25 Richmond, CA) to which recombinant human IL-1 α had been coupled. ^{125}I -labeled receptor was then eluted with sample buffer (0.0625 M Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol) and analyzed by SDS polyacrylamide gel electrophoresis on a 10% gel. Gels were then subjected to autoradiography. The recombinant IL-1 receptor
30 purified by affinity chromatography on IL-1 α columns migrated with a relative mobility of about 80,000 on SDS polyacrylamide gels, comparable to the mobility displayed by IL-1 receptor purified in the same manner from EL-4 6.1 C10 cells.

The DNA from clone 78, when transfected into COS cells, led
35 to expression of IL-1 binding activity which was virtually identical

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to that displayed by EL-4 6.1 C10 cells, as shown in Figures 3A-3C.

For binding assays, COS cells were resuspended at 1.7×10^6 cells/ml with EL-4 M (1.5×10^7 cells/ml) cells as carriers. EL-4 M and EL-4 6.1 C10 were resuspended at 1.5×10^7 cells/ml. All cell
5 suspensions were made and binding assays done in RPMI 1640/10% BSA/0.1% sodium azide/20 mM HEPES pH 7.4. Binding incubations with ^{125}I -IL-1 α or ^{125}I -IL-1 β and unlabeled IL-1 α and IL-1 β were done as described elsewhere in the specification. ^{125}I -IL-1 α bound to the transfected COS cells with a K_d of $3.0 \pm 0.2 \times 10^9 \text{ M}^{-1}$ (Figure 3B).
10 The K_d for the native receptor on EL-4 6.1 C10 cells was $4.3 \pm 3 \times 10^9 \text{ M}^{-1}$. All of the binding was to recombinant receptors (see Figure 3A); the parental COS cell population did not bind detectable ^{125}I -IL-1 α in this experiment.

In a cold competition experiment, free ^{125}I -IL-1 α
15 concentration was $7.72 \pm 0.13 \times 10^{-10} \text{ M}$. On the transfected COS cells the maximal binding was $2.98 \pm 0.3 \times 10^4$ molecules/cell (no inhibition) and the background (measured in the presence of $6 \times 10^{-7} \text{ M}$ unlabeled IL-1 α) was 921 ± 60 molecules/cell (100% inhibition). On the EL-4 6.1 C10 cells maximal binding was $1.33 \pm 0.02 \times 10^4$
20 molecules/cell and background (see above) was 47 ± 2 molecules/cell. Binding of ^{125}I -IL-1 α , both to the transfected COS cells and to EL-4 6.1 C10 cells, could be competed completely by an excess of either unlabeled IL-1 α or unlabeled IL-1 β (Figure 3C). The inhibition constants for IL-1 α and for IL-1 β were very similar with each cell
25 type (Figure 3C).

Example 7

Preparation of Monoclonal Antibodies to IL-1R

30 Preparations of purified recombinant IL-1R, for example, human IL-1R, or transfected COS cells expressing high levels of IL-1R are employed to generate monoclonal antibodies against IL-1R using conventional techniques, for example, those disclosed in U. S. Patent 4,411,993. Such antibodies are likely to be useful in interfering
35 with IL-1 binding to IL-1 receptors, for example, in ameliorating

toxic or other undesired effects of IL-1.

To immunize mice, IL-1R immunogen is emulsified in complete Freund's adjuvant and injected in amounts ranging from 10-100 µg subcutaneously into Balb/c mice. Ten to twelve days later, the immunized animals are boosted with additional immunogen emulsified in incomplete Freund's adjuvant and periodically boosted thereafter on a weekly to biweekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay, ELISA (enzyme-linked immunosorbent assay), or inhibition of binding of ^{125}I -IL-1 α to extracts of EL-4 6.1 C10 cells (as described above). Other assay procedures are also suitable. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to the murine myeloma cell line NS1. Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with IL-1R, for example, by adaptations of the techniques disclosed by Engvall et al., Immunochemistry 8:871 (1971) and in U. S. Patent 4,703,004. Positive clones are then injected into the peritoneal cavities of syngeneic Balb/c mice to produce ascites containing high concentrations (>1 mg/ml) of anti-IL-1R monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein A of Staphylococcus aureus.

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Example 8

Expression of IL-1R in Yeast

For expression of human or murine IL-1R in yeast, a yeast expression vector derived from pIXY120 is constructed as follows.

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pIXY120 is identical to pY α HuGM (ATCC 53157), except that it contains no cDNA insert and includes a polylinker/multiple cloning site with an NcoI site. This vector includes DNA sequences from the following sources: (1) a large SphI (nucleotide 562) to EcoRI (nucleotide 4361) fragment excised from plasmid pBR322 (ATCC 37017), including the origin of replication and the ampicillin resistance marker for selection in *E. coli*; (2) *S. cerevisiae* DNA including the TRP-1 marker, 2 μ origin of replication, ADH2 promoter; and (3) DNA encoding an 85 amino acid signal peptide derived from the gene encoding the secreted peptide α -factor (See Kurjan et al., U.S. Patent 4,546,082). An Asp718 restriction site was introduced at position 237 in the α -factor signal peptide to facilitate fusion to heterologous genes. This was achieved by changing the thymidine residue at nucleotide 241 to a cytosine residue by oligonucleotide-directed *in vitro* mutagenesis as described by Craik, *Biotechniques*:12 (1985). A synthetic oligonucleotide containing multiple cloning sites and having the following sequence was inserted from the Asp718 site at amino acid 79 near the 3' end of the α -factor signal peptide to a SpeI site in the 2 μ sequence:

```

20      Asp718                               StuI  NcoI  BamHI
      GTACCTTTGGATAAAAGAGACTACAAGGACGACGATGACAAGAGGCCTCCATGGAT...
      GAAACCTATTTTCTCTGATGTTCTGCTGCTACTGTTCTCCGGAGGTACCTA...
                                   |←----Polylinker--

      SmaI           SpeI
25      ...CCCCCGGGACA
      ...GGGGGCCCTGTGATC
      ---Polylinker--->|

```

pBC120 also varies from pY α HuGM by the presence of a 514 bp DNA fragment derived from the single-standed phage f1 containing the origin of replication and intergenic region, which has been inserted at the NruI site in the pBR322 sequence. The presence of an f1 origin of replication permits generation of single-stranded DNA copies of the vector when transformed into appropriate strains of *E. coli* and superinfected with bacteriophage f1, which facilitates DNA sequencing of the vector and provides a basis for *in vitro* mutagenesis. To

insert a cDNA, pIXY120 is digested with Asp718 which cleaves near the 3' end of the α -factor leader peptide (nucleotide 237) and, for example, NcoI which cleaves in the polylinker. The large vector fragment is then purified and ligated to a DNA fragment encoding the protein to be expressed.

To create a secretion vector for expressing human IL-1R, a cDNA fragment including the complete open reading frame encoding hIL-1R is cleaved with an appropriate restriction endonuclease proximal to the N-terminus of the mature protein. An oligonucleotide or oligonucleotides are then synthesized which are capable of ligation to the 5' and 3' ends of the hIL-1R fragment, regenerating any codons deleted in isolating the fragment, and also providing cohesive termini for ligation to pIXY120 to provide a coding sequence located in frame with respect to an intact α -factor leader sequence.

The resulting expression vectors are then purified and employed to transform a diploid yeast strain of *S. cerevisiae* (XV2181) by standard techniques, such as those disclosed in EPA 0165654, selecting for tryptophan prototrophs. The resulting transformants are cultured for expression of an hIL-1R protein as a secreted or extracted product. Cultures to be assayed for hIL-1R expression are grown in 20-50 ml of YPD medium (1% yeast extract, 2% peptone, 1% glucose) at 37°C to a cell density of $1-5 \times 10^8$ cells/ml. To separate cells from medium, cells are removed by centrifugation and the medium filtered through a 0.45 μ cellulose acetate filter prior to assay. Supernatants produced by the transformed yeast strain, or extracts prepared from disrupted yeast cells, are assayed for the presence of hIL-1R using binding assays as described above.

Example 9

Construction, Expression and Purification of Truncated Recombinant Murine IL-1 Receptor

A truncated version of the IL-1 receptor protein was produced using an expression system compatible with the HELA-EBNA1 cell line, which constitutively expresses Epstein-Barr virus nuclear antigen driven from the CMV immediate-early enhancer promoter. The expression

vector used was termed HAV-E0, a derivative of pDC201 which contains the Epstein-Barr virus origin and allows high level expression in the HELA-EBNA cell line. HAV-E0 is derived from pDC201 by replacement of the adenovirus major late promoter with synthetic sequences from HIV-1
5 extending from the cap site of the viral mRNA, using the SV-40 early promoter to drive expression of the HIV-1 tat gene.

The expression construct for the soluble truncated IL-1 receptor was generated in a series of steps. The entire coding region of the receptor and part of the 5' untranslated region were removed
10 from the original IL-1 receptor clone 78 by digestion with Asp 718 and NdeI. This fragment, containing no 3' untranslated sequences, was cloned into HAV-E0, to generate HAV-E0-FL9. A variant of this plasmid, containing a translational stop codon immediately following the codon for proline 316 and lacking all the coding sequence 3' to
15 this, was subsequently constructed by standard methods and termed HAV-E0-MEXT.

HAV-E0-MEXT vector DNA was introduced into HELA-EBNA cells by a modified polybrene transfection as disclosed by Kawai and Nishizawa (Mol. Cell Biol. 4:1172, 1984). 1.5×10^6 cells were seeded into 10
20 ml DMEM + 10% FCS, in a 10 cm tissue culture dish. Cells were incubated at 37°C, 10% CO₂ for 16 hours. The media was then removed and 3 ml of serum-free DMEM containing 10 µg/ml DNA and 30 µg/ml polybrene (Sigma) were added. Dishes were then incubated at 37°C/10% CO₂ for a further six hours, at which time the DNA mix was removed and
25 cells were glycerol shocked by addition of 3 ml serum-free DMEM + 25% glycerol (v/v) for one minute. Glycerol was removed, and the cells were washed twice with medium. Ten ml of DMEM + 10% FCS were then added, and the cells were incubated at 37°C/10% CO₂ for 18 hours.

Transfected cells were then removed with trypsin and split in
30 a ratio of 1:9 into T175 cm² flasks (to give approximately 10% confluence) containing 25 ml DMEM + 1% FCS. Supernatants containing transiently expressed soluble murine IL-1 receptor were harvested every 24 hours for up to ten days.

IL-1α binding activity in the medium was measured by
35 inhibition of ¹²⁵I-IL-1α to EL4 6.1 C10 cells as described by Mosley et

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al. (J. Biol. Chem. 262:2941, 1987) with the exception that labeled IL-1 α (2×10^{-11} , 50 μ l was first incubated with the test sample (50 μ l) for two hours at 8°C, prior to addition of cells (2.5×10^6 cells, 50 μ l). Each test sample was assayed at six dilutions (X3) and the inhibition dose response curve used to assess the relative inhibitory titer.

Soluble IL-1 receptor was purified from culture supernatants as described for natural receptor by Urdal et al. (J. Biol. Chem. 263:280, 1988). Culture supernatants were passed over a 1 ml bed volume IL-1 α column, the column was washed with PBS and eluted with 0.1 M glycine-HCl. Acid eluate fractions were immediately neutralized and subsequently tested for IL-1 binding activity using the radioreceptor inhibition assay. SDS-polyacrylamide gel electrophoresis of the material eluted by the acid treatment showed that it contained two bands of M_r 60,000 and 54,000. N-glycanase treatment of this material indicated that the size heterogeneity is due to differences in N-linked glycosylation between the two species. Soluble IL-1 receptor retains full IL-1 binding activity.

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TABLE 1: cDNA SEQUENCE OF IL-1R CLONE IN GEMBL78

1	5'-TGGGTCGTCT	GACTAGAAGT	GAGCTGTCTG	TCATTCTTGT	GCACGCCAGC
51	CCAGTAATCA	TTTGGAGGCA	AAGCAAACCTG	TAAGTAATGC	TGTCCTGGGC
101	TGACTTGAGG	AGGCAGTTTT	CGTTTTAACA	GCCAGTGTTT	ATTTGCTCAG
151	CAAACGTTGT	CTCGGGGAGA	AATGTCGCTG	GATGTCATCA	GAGTTCCCAG
201	TGCCCCGAAC	CGTGAACAAC	ACAAATGGAG	AATATGAAAG	TGCTACTGGG
251	GCTCATTTGT	CTCATGGTGC	CTCTGCTGTC	GCTGGAGATT	GACGTATGTA
301	CAGAATATCC	AAATCAGATC	GTTTTGTTTT	TATCTGTAAA	TGAAATTGAT
351	ATTGCGAAGT	GTCCTCTTAC	TCCAAATAAA	ATGCACGGCG	ACACCATAAT
401	TTGGTACAAG	AATGACAGCA	AGACCCCAT	ATCAGCGGAC	CGGGACTCCA
451	GGATTCATCA	GCAGAATGAA	CATCTTTGGT	TTGTACCTGC	CAAGGTGGAG
501	GACTCAGGAT	ATTACTATTG	TATAGTAAGA	AACTCAACTT	ACTGCCTCAA
551	AACTAAAGTA	ACCGTAACTG	TGTTAGAGAA	TGACCCTGGC	TTGTGTTACA
601	GCACACAGGC	CACCTTCCCA	CAGCGGCTCC	ACATTGCCGG	GGATGGAAGT
651	CTTGTGTGCC	CTTATGTGAG	TTATTTTAAA	GATGAAAATA	ATGAGTTACC
701	CGAGGTCCAG	TGGTATAAGA	ACTGTAAACC	TCTGCTTCTT	GACAACGTGA
751	GCTTCTTCGG	AGTAAAAGAT	AAACTGTTGG	TGAGGAATGT	GGCTGAAGAG
801	CACAGAGGGG	ACTATATATG	CCGTATGTCC	TATACGTTCC	GGGGGAAGCA
851	ATATCCGGTC	ACACGAGTAA	TACAATTTAT	CACAATAGAT	GAAAACAAGA
901	GGGACAGACC	TGTTATCCTG	AGCCCTCGGA	ATGAGACGAT	CGAAGCTGAC
951	CCAGGATCAA	TGATACAAC	GATCTGCAAC	GTCACGGGCC	AGTTCTCAGA
1001	CCTTGTCTAC	TGGAAGTGGA	ATGGATCAGA	AATTGAATGG	AATGATCCAT
1051	TTCTAGCTGA	AGACTATCAA	TTTGTGGAAC	ATCCTTCAAC	CAAAAGAAAA
1101	TACACACTCA	TTACAACACT	TAACATTTCA	GAAGTTAAAA	GCCAGTTTTA
1151	TCGCTATCCG	TTTATCTGTG	TTGTTAAGAA	CACAAATATT	TTTGAGTCGG
1201	CGCATGTGCA	GTTAATATAC	CCAGTCCCTG	ACTTCAAGAA	TTACCTCATC
1251	GGGGGCTTTA	TCATCCTCAC	GGCTACAATT	GTATGCTGTG	TGTGCATCTA
1301	TAAAGTCTTC	AAGGTTGACA	TAGTGCTTTG	GTACAGGGAC	TCCTGCTCTG
1351	GTTTTCTTCC	TTCAAAAGCT	TCAGATGGAA	AGACATACGA	TGCCTATATT
1401	CTTTATCCCA	AGACCCTGGG	AGAGGGGTCC	TTCTCAGACT	TAGATACTTT
1451	TGTTTTTAAA	CTGTTGCCTG	AGGTCTTGGA	GGGACAGTTT	GGATACAAGC
1501	TGTTCAATTA	TGGAAGGGAT	GACTATGTTG	GAGAAGATAC	CATCGAGGTT
1551	ACTAATGAAA	ATGTAAAGAA	AAGCAGGAGG	CTGATTATCA	TTCTAGTGAG
1601	AGATATGGGA	GGCTTCAGCT	GGCTGGGCCA	GTCATCTGAA	GAGCAAATAG
1651	CCATATACAA	TGCTCTCATC	CAGGAAGGAA	TTAAAAATCGT	CCTGCTTGAG
1701	TTGGAGAAAA	TCCAAGACTA	TGAGAAAAATG	CCAGATTCTA	TTCAGTTCAT
1751	TAAGCAGAAA	CACGGAGTCA	TTTGCTGGTC	AGGAGACTTT	CAAGAAAAGAC
1801	CACAGTCTGC	AAAGACCAGG	TTCTGGAAAA	ACTTAAGATA	CCAGATGCCA
1851	GCCCAACGGA	GATCACCATT	GTCTAAACAC	CGCTTACTAA	CCCTGGATCC
1901	TGTGCGGGAC	ACTAAGGAGA	AACTGCCGGC	AGCAACACAC	TTACCACTCG
1951	GCTAGCATGG	CAAAAGTGGG	CAGGCCAAGA	ACTTCGGAAT	ATCTCCCATC
2001	ATAAGAGGCT	GCAGCTGGGC	TGTGCCTCCC	AGTAAAACAG	TCACGAACCA
2051	AACCTGTGCA	GTCCCTTGTT	CCAGATCACC	TGGAACCTGGA	TTGGGAAGAG
2101	AACAGGACTT	GGTGGCCAGG	ACCGCTCAGA	GAGCCATGGT	TGCTCAGGGA
2151	TGCTGCTCCG	GGATGCTTGA	CTAACAGTCG	AGGCAGTGAA	CTGGGTGTAG
2201	AAAGCGTCAG	GAAATGGCCA	CATGTGTGGA	TGGTTTAATT	AGATTCTGTG
2251	GAGTCTCACA	GTGGGATTGT	GGCTGTCTGA	GGACACTTTG	GGGGGTCGCT
2301	GTCCAAGAAG	TGGCTCCCCA	AAGTATAAGT	GCGGGTGAGG	TTTACTGATA
2351	CCCCAC-3'				

TABLE 2A: Sequence of Coding Region of Murine IL-1 Receptor Gene

5'-ATG	GAG	AAT	ATG	AAA	GTG	CTA	CTG	GGG	CTC	ATT	TGT	CTC	ATG	GTG	-15
<u>Met</u>	Glu	Asn	<u>Met</u>	Lys	Val	Leu	Leu	Gly	Leu	Ile	Cys	Leu	Met	Val	-5
CCT	CTG	CTG	TCG	CTG	GAG	ATT	GAC	GTA	TGT	ACA	GAA	TAT	CCA	AAT	33
Pro	Leu	Leu	Ser	<u>Leu</u>	Glu	Ile	Asp	Val	Cys	Thr	Glu	Tyr	Pro	Asn	11
CAG	ATC	GTT	TTG	TTT	TTA	TCT	GTA	AAT	GAA	ATT	GAT	ATT	CGC	AAG	78
Gln	Ile	Val	Leu	Phe	Leu	Ser	Val	Asn	Glu	Ile	Asp	Ile	Arg	Lys	26
TGT	CCT	CTT	ACT	CCA	AAT	AAA	ATG	CAC	GGC	GAC	ACC	ATA	ATT	TGG	123
Cys	Pro	Leu	Thr	Pro	Asn	Lys	Met	His	Gly	Asp	Thr	Ile	Ile	Trp	41
TAC	AAG	AAT	GAC	AGC	AAG	ACC	CCC	ATA	TCA	GCG	GAC	CGG	GAC	TCC	168
Tyr	Lys	Asn	Asp	Ser	Lys	Thr	Pro	Ile	Ser	Ala	Asp	Arg	Asp	Ser	56
AGG	ATT	CAT	CAG	CAG	AAT	GAA	CAT	CTT	TGG	TTT	GTA	CCT	GCC	AAG	213
Arg	Ile	His	Gln	Gln	Asn	Glu	His	Leu	Trp	Phe	Val	Pro	Ala	Lys	71
GTG	GAG	GAC	TCA	GGA	TAT	TAC	TAT	TGT	ATA	GTA	AGA	AAC	TCA	ACT	258
Val	Glu	Asp	Ser	Gly	Tyr	Tyr	Tyr	Cys	Ile	Val	Arg	Asn	Ser	Thr	86
TAC	TGC	CTC	AAA	ACT	AAA	GTA	ACC	GTA	ACT	GTG	TTA	GAG	AAT	GAC	303
Tyr	Cys	Leu	Lys	Thr	Lys	Val	Thr	Val	Thr	Val	Leu	Glu	Asn	Asp	101
CCT	GGC	TTG	TGT	TAC	AGC	ACA	CAG	GCC	ACC	TTC	CCA	CAG	CGG	CTC	348
Pro	Gly	Leu	Cys	Tyr	Ser	Thr	Gln	Ala	Thr	Phe	Pro	Gln	Arg	Leu	116
CAC	ATT	GCC	GGG	GAT	GGA	AGT	CTT	GTG	TGC	CCT	TAT	GTG	AGT	TAT	393
His	Ile	Ala	Gly	Asp	Gly	Ser	Leu	Val	Cys	Pro	Tyr	Val	Ser	Tyr	131
TTT	AAA	GAT	GAA	AAT	AAT	GAG	TTA	CCC	GAG	GTC	CAG	TGG	TAT	AAG	438
Phe	Lys	Asp	Glu	Asn	Asn	Glu	Leu	Pro	Glu	Val	Gln	Trp	Tyr	Lys	146
AAC	TGT	AAA	CCT	CTG	CTT	CTT	GAC	AAC	GTG	AGC	TTC	TTC	GGA	GTA	483
Asn	Cys	Lys	Pro	Leu	Leu	Leu	Asp	Asn	Val	Ser	Phe	Phe	Gly	Val	161
AAA	GAT	AAA	CTG	TTG	GTG	AGG	AAT	GTG	GCT	GAA	GAG	CAC	AGA	GGG	528
Lys	Asp	Lys	Leu	Leu	Val	Arg	Asn	Val	Ala	Glu	Glu	His	Arg	Gly	176
GAC	TAT	ATA	TGC	CGT	ATG	TCC	TAT	ACG	TTC	CGG	GGG	AAG	CAA	TAT	573
Asp	Tyr	Ile	Cys	Arg	Met	Ser	Tyr	Thr	Phe	Arg	Gly	Lys	Gln	Tyr	191
CCG	GTC	ACA	CGA	GTA	ATA	CAA	TTT	ATC	ACA	ATA	GAT	GAA	AAC	AAG	618
Pro	Val	Thr	Arg	Val	Ile	Gln	Phe	Ile	Thr	Ile	Asp	Glu	Asn	Lys	206

TABLE 2B: Sequence of Coding Region of Murine IL-1 Receptor Gene

AGG GAC AGA CCT GTT ATC CTG AGC CCT CGG AAT GAG ACG ATC GAA	663
Arg Asp Arg Pro Val Ile Leu Ser Pro Arg Asn Glu Thr Ile Glu	221
GCT GAC CCA GGA TCA ATG ATA CAA CTG ATC TGC AAC GTC ACG GGC	708
Ala Asp Pro Gly Ser Met Ile Gln Leu Ile Cys Asn Val Thr Gly	236
CAG TTC TCA GAC CTT GTC TAC TGG AAG TGG AAT GGA TCA GAA ATT	753
Gln Phe Ser Asp Leu Val Tyr Trp Lys Trp Asn Gly Ser Glu Ile	251
GAA TGG AAT GAT CCA TTT CTA GCT GAA GAC TAT CAA TTT GTG GAA	798
Glu Trp Asn Asp Pro Phe Leu Ala Glu Asp Tyr Gln Phe Val Glu	266
CAT CCT TCA ACC AAA AGA AAA TAC ACA CTC ATT ACA ACA CTT AAC	843
His Pro Ser Thr Lys Arg Lys Tyr Thr Leu Ile Thr Thr Leu Asn	281
ATT TCA GAA GTT AAA AGC CAG TTT TAT CGC TAT CCG TTT ATC TGT	888
Ile Ser Glu Val Lys Ser Gln Phe Tyr Arg Tyr Pro Phe Ile Cys	296
GTT GTT AAG AAC ACA AAT ATT TTT GAG TCG GCG CAT GTG CAG TTA	933
Val Val Lys Asn Thr Asn Ile Phe Glu Ser Ala His Val Gln Leu	311
ATA TAC CCA GTC CCT GAC TTC AAG AAT TAC CTC ATC GGG GGC TTT	978
Ile Tyr Pro Val Pro Asp Phe Lys <u>Asn Tyr Leu Ile Gly Gly Phe</u>	326
ATC ATC CTC ACG GCT ACA ATT GTA TGC TGT GTG TGC ATC TAT AAA	1023
<u>Ile Ile Leu Thr Ala Thr Ile Val Cys Cys Val Cys Ile Tyr Lys</u>	341
GTC TTC AAG GTT GAC ATA GTG CTT TGG TAC AGG GAC TCC TGC TCT	1068
Val Phe Lys Val Asp Ile Val Leu Trp Tyr Arg Asp Ser Cys Ser	356
GGT TTT CTT CCT TCA AAA GCT TCA GAT GGA AAG ACA TAC GAT GCC	1113
Gly Phe Leu Pro Ser Lys Ala Ser Asp Gly Lys Thr Tyr Asp Ala	371
TAT ATT CTT TAT CCC AAG ACC CTG GGA GAG GGG TCC TTC TCA GAC	1158
Tyr Ile Leu Tyr Pro Lys Thr Leu Gly Glu Gly Ser Phe Ser Asp	386
TTA GAT ACT TTT GTT TTT AAA CTG TTG CCT GAG GTC TTG GAG GGA	1203
Leu Asp Thr Phe Val Phe Lys Leu Leu Pro Glu Val Leu Glu Gly	401
CAG TTT GGA TAC AAG CTG TTC ATT TAT GGA AGG GAT GAC TAT GTT	1248
Gln Phe Gly Tyr Lys Leu Phe Ile Tyr Gly Arg Asp Asp Tyr Val	416
GGA GAA GAT ACC ATC GAG GTT ACT AAT GAA AAT GTA AAG AAA AGC	1293
Gly Glu Asp Thr Ile Glu Val Thr Asn Glu Asn Val Lys Lys Ser	431

TABLE 2C: Sequence of Coding Region of Murine IL-1 Receptor Gene

AGG AGG CTG ATT ATC ATT CTA GTG AGA GAT ATG GGA GGC TTC AGC	1338
Arg Arg Leu Ile Ile Ile Leu Val Arg Asp Met Gly Gly Phe Ser	446
TGG CTG GGC CAG TCA TCT GAA GAG CAA ATA GCC ATA TAC AAT GCT	1383
Trp Leu Gly Gln Ser Ser Glu Glu Gln Ile Ala Ile Tyr Asn Ala	461
CTC ATC CAG GAA GGA ATT AAA ATC GTC CTG CTT GAG TTG GAG AAA	1428
Leu Ile Gln Glu Gly Ile Lys Ile Val Leu Leu Glu Leu Glu Lys	476
ATC CAA GAC TAT GAG AAA ATG CCA GAT TCT ATT CAG TTC ATT AAG	1473
Ile Gln Asp Tyr Glu Lys Met Pro Asp Ser Ile Gln Phe Ile Lys	491
CAG AAA CAC GGA GTC ATT TGC TGG TCA GGA GAC TTT CAA GAA AGA	1518
Gln Lys His Gly Val Ile Cys Trp Ser Gly Asp Phe Gln Glu Arg	506
CCA CAG TCT GCA AAG ACC AGG TTC TGG AAA AAC TTA AGA TAC CAG	1563
Pro Gln Ser Ala Lys Thr Arg Phe Trp Lys Asn Leu Arg Tyr Gln	521
ATG CCA GCC CAA CGG AGA TCA CCA TTG TCT AAA CAC CGC TTA CTA	1608
Met Pro Ala Gln Arg Arg Ser Pro Leu Ser Lys His Arg Leu Leu	536
ACC CTG GAT CCT GTG CGG GAC ACT AAG GAG AAA CTG CCG GCA GCA	1653
Thr Leu Asp Pro Val Arg Asp Thr Lys Glu Lys Leu Pro Ala Ala	551
ACA CAC TTA CCA CTC GGC TAG-3'	1671
Thr His Leu Pro Leu Gly End	557

TABLE 3: cDNA SEQUENCE OF HUMAN IL-1R CONSTRUCT

1	5'-AGACGCACCC	TCTGAAGATG	GTGGACTCCC	TCCTGAGAAG	CTGGGACCCC
51	TTGGTAAAAG	ACAAGGCCTT	CTCCAAGAAG	AATATGAAAG	TGTTACTCAG
101	ACTTATTTGT	TTCATAGCTC	TACTGATTTT	TTCTCTGGAG	GCTGATAAAT
151	GCAAGGAACG	TGAAGAAAAA	ATAATTTTAT	TGTCATCTGC	AAATGAAATT
201	GATGTTTCGTC	CCTGTCCTCT	TAACCCAAAT	GAACACAAAG	GCACTATAAC
251	TTGGTATAAA	GATGACAGCA	AGACACCTGT	ATCTACAGAA	CAAGCCTCCA
301	GGATTCATCA	ACACAAAGAG	AAACTTTGGT	TTGTTCCCTGC	TAAGGTGGAG
351	GATTCAGGAC	ATTACTATTG	CGTGGTAAGA	AATTCATCTT	ACTGCCTCAG
401	AATTAAAATA	AGTGCAAAAT	TTGTGGAGAA	TGAGCCTAAC	TTATGTTATA
451	ATGCACAAGC	CATATTTAAG	CAGAACTAC	CCGTTGCAGG	AGACGGAGGA
501	CTTGTGTGCC	CTTATATGGA	GTTTTTTAAA	AATGAAAATA	ATGAGTTACC
551	TAAATTACAG	TGGTATAAGG	ATTGCAAACC	TCTACTTCTT	GACAATATAC
601	ACTTTAGTGG	AGTCAAAGAT	AGGCTCATCG	TGATGAATGT	GGCTGAAAAG
651	CATAGAGGGA	ACTATACTTG	TCATGCATCC	TACACATACT	TGGGCAAGCA
701	ATATCCTATT	ACCCGGGTAA	TAGAATTTAT	TACTCTAGAG	GAAAACAAAC
751	CCACAAGGCC	TGTGATTGTG	AGCCCAGCTA	ATGAGACAAT	GGAAGTAGAC
801	TTGGGATCCC	AGATACAATT	GATCTGTAAT	GTCACCGGCC	AGTTGAGTGA
851	CATTGCTTAC	TGGAAGTGGA	ATGGGTCAGT	AATTGATGAA	GATGACCCAG
901	TGCTAGGGGA	AGACTATTAC	AGTGTGGAAG	ATCCTGCAAA	CAAAAGAAGG
951	AGTACCCTCA	TCACAGTGCT	TAATATATCG	GAAATTGAAA	GTAGATTTTA
1001	TAAACATCCA	TTTACCTGTT	TTGCCAAGAA	TACACATGGT	ATAGATGCAG
1051	CATATATCCA	GTTAATATAT	CCAGTCACTA	ATTTCCAGAA	GCACATGATT
1101	GGTATATGTG	TCACGTTGAC	AGTCATAATT	GTGTGTTCTG	TTTTCATCTA
1151	TAAAATCTTC	AAGATTGACA	TTGTGCTTTG	GTACAGGGAT	TCCTGCTATG
1201	ATTTTCTCCC	AATAAAAGCT	TCAGATGGAA	AGACCTATGA	CGCATATATA
1251	CTGTATCCAA	AGACTGTTGG	GGAAGGGTCT	ACCTCTGACT	GTGATATTTT
1301	TGTGTTTTAA	GTCTTGCCCTG	AGGTCTTGGA	AAAACAGTGT	GGATATAAGC
1351	TGTTCAATTA	TGGAAGGGAT	GACTACGTTG	GGGAAGACAT	TGTTGAGGTC
1401	ATTAATGAAA	ACGTAAAGAA	AAGCAGAAGA	CTGATTATCA	TTTGTAGTCAG
1451	AGAAACATCA	GGCTTCAGCT	GGCTGGGTGG	TTTATCTGAA	GAGCAAAATAG
1501	CCATGTATAA	TGCTCTTGTT	CAGGATGGAA	TTAAAGTTGT	CCTGCTTGAG
1551	CTGGAGAAAA	TCCAAGACTA	TGAGAAAAATG	CCAGAATCGA	TTAAATTCAT
1601	TAAGCAGAAA	CATGGGGCTA	TCCGCTGGTC	AGGGGACTTT	ACACAGGGAC
1651	CACAGTCTGC	AAAGACAAGG	TCTGGAAGA	ATGTCAGGTA	CCACATGCCA
1701	GTCCAGCGAC	GGTCACCTTC	ATCTAAACAC	CAGTTACTGT	CACCAGCCAC
1751	TAAGGAGAAA	CTGCAAAGAG	AGGCTCACGT	GCCTCTCGGG	TAGCATGGAG
1801	AAGTTGCCAA	GAGTTCCTTA	GGTGCCCTCT	GTCTTATGGC	GTTGCAGGCC
1851	AGGTTATGCC	TCATGCTGAC	TTGCAGAGTT	CATGGAATGT	AACTATATCA
1901	TCCTTTATCC	CTGAGGTCAC	CTGGAATCAG	ATTATTAAGG	GAATAAGCCA
1951	TGACGTCAAT	AGCAGCCCAG	GGCACTTCAG	AGTAGAGGGC	TTGGGAAGAT
2001	CTTTTAAAAA	GGCAGTAGGC	CCGGTGTTGGT	GGCTCACGCC	TATAATCCCA
2051	GCACTTTGGG	AGGCTGAAGT	GGGTGGATCA	CCAGAGGTCA	GGAGTTCGAG
2101	ACCAGCCCAG	CCAACATGGC	AAAACCCCAT	CTCTACTAAA	AATACAAAAA
2151	TGAGCTAGGC	ATGGTGGCAC	ACGCCTGTAA	TCCCAGCTAC	ACCTGAGGCT
2201	GAGGCAGGAG	AATTGCTTGA	ACCGGGGAGA	CGGAGGTTGC	AGTGAGCCGA
2251	GTTTGGGCCA	CTGCACTCTA	GCCTGGCAAC	AGAGCAAGAC	TCCGTCTCAA
2301	AAAAAGGGCA	ATAAATGCCC	TCTCTGAATG	TTTGAAGTGC	CAAGAAAAGG
2351	CATGGAGACA	GCGAACTAGA	AGAAAGGGCA	AGAAGGAAAT	AGCCACCGTC
2401	TACAGATGGC	TTAGTTAAGT	CATCCACAGC	CCAAGGGCGG	CGGCTATGCC
2451	TTGTCTGGGG	ACCCTGTAGA	GTCCTGACC	CTGGAGCGGC	TCTCCTGAGA
2501	GGTGCTGCAG	GCAAAGTGAG	ACTGACACCT	CACTGAGGAA	GGGAGACATA
2551	TTCTTGAGAG	ACTTCCATC	TGCTTGATAT	TTCCATACAC	ATCCCCAGCC-3'

TABLE 4A: Sequence of Coding Region of Human IL-1 Receptor Gene

ATG AAA GTG TTA CTC AGA CTT ATT TGT TTC ATA GCT CTA CTG ATT	-9
Met Lys Val Leu Leu Arg Leu Ile Cys Phe Ile Ala Leu Leu Ile	-3
TCT TCT CTG GAG GCT GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA	39
Ser Ser <u>Leu</u> Glu Ala Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile	13
ATT TTA GTG TCA TCT GCA AAT GAA ATT GAT GTT CGT CCC TGT CCT	84
Ile Leu Val Ser Ser Ala Asn Glu Ile Asp Val Arg Pro Cys Pro	28
CTT AAC CCA AAT GAA CAC AAA GGC ACT ATA ACT TGG TAT AAA GAT	129
Leu Asn Pro Asn Glu His Lys Gly Thr Ile Thr Trp Tyr Lys Asp	43
GAC AGC AAG ACA CCT GTA TCT ACA GAA CAA GCC TCC AGG ATT CAT	174
Asp Ser Lys Thr Pro Val Ser Thr Glu Gln Ala Ser Arg Ile His	58
CAA CAC AAA GAG AAA CTT TGG TTT GTT CCT GCT AAG GTG GAG GAT	219
Gln His Lys Glu Lys Leu Trp Phe Val Pro Ala Lys Val Glu Asp	73
TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT TCA TCT TAC TGC CTC	264
Ser Gly His Tyr Tyr Cys Val Val Arg Asn Ser Ser Tyr Cys Leu	88
AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT GAG CCT AAC TTA	309
Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn Glu Pro Asn Leu	103
TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA CCC GTT GCA	354
Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu Pro Val Ala	118
GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT AAA AAT	399
Gly Asp Gly Gly Leu Val Cys Pro Tyr Met Glu Phe Phe Lys Asn	133
GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC AAA	444
Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys Lys	148
CCT CTA CTT CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG	489
Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg	163
CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT	534
Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr	178
TGT CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC	579
Cys His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr	193
CGG GTA ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG	624
Arg Val Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg	208
CCT GTG ATT GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG	669
Pro Val Ile Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu	223

TABLE 4B: Sequence of Coding Region of Human IL-1 Receptor Gene

GGA TCC CAG ATA CAA TTG ATC TGT AAT GTC ACC GGC CAG TTG AGT	714
Gly Ser Gln Ile Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser	238
GAC ATT GCT TAC TGG AAG TGG AAT GGG TCA GTA ATT GAT GAA GAT	759
Asp Ile Ala Tyr Trp Lys Trp Asn Gly Ser Val Ile Asp Glu Asp	253
GAC CCA GTG CTA GGG GAA GAC TAT TAC AGT GTG GAA AAT CCT GCA	804
Asp Pro Val Leu Gly Glu Asp Tyr Tyr Ser Val Glu Asn Pro Ala	268
AAC AAA AGA AGG AGT ACC CTC ATC ACA GTG CTT AAT ATA TCG GAA	849
Asn Lys Arg Arg Ser Thr Leu Ile Thr Val Leu Asn Ile Ser Glu	283
ATT GAA AGT AGA TTT TAT AAA CAT CCA TTT ACC TGT TTT GCC AAG	894
Ile Glu Ser Arg Phe Tyr Lys His Pro Phe Thr Cys Phe Ala Lys	298
AAT ACA CAT GGT ATA GAT GCA GCA TAT ATC CAG TTA ATA TAT CCA	939
Asn Thr His Gly Ile Asp Ala Ala Tyr Ile Gln Leu Ile Tyr Pro	313
GTC ACT AAT TTC CAG AAG CAC ATG ATT GGT ATA TGT GTC ACG TTG	984
Val Thr Asn Phe Gln Lys <u>His Met Ile Gly Ile Cys Val Thr Leu</u>	328
ACA GTC ATA ATT GTG TGT TCT GTT TTC ATC TAT AAA ATC TTC AAG	1029
Thr Val Ile Ile Val Cys Ser Val Phe Ile Tyr Lys Ile Phe Lys	343
ATT GAC ATT GTG CTT TGG TAC AGG GAT TCC TGC TAT GAT TTT CTC	1074
Ile Asp Ile Val Leu Trp Tyr Arg Asp Ser Cys Tyr Asp Phe Leu	358
CCA ATA AAA GCT TCA GAT GGA AAG ACC TAT GAC GCA TAT ATA CTG	1119
Pro Ile Lys Ala Ser Asp Gly Lys Thr Tyr Asp Ala Tyr Ile Leu	373
TAT CCA AAG ACT GTT GGG GAA GGG TCT ACC TCT GAC TGT GAT ATT	1164
Tyr Pro Lys Thr Val Gly Glu Gly Ser Thr Ser Asp Cys Asp Ile	388
TTT GTG TTT AAA GTC TTG CCT GAG GTC TTG GAA AAA CAG TGT GGA	1209
Phe Val Phe Lys Val Leu Pro Glu Val Leu Glu Lys Gln Cys Gly	403
TAT AAG CTG TTC ATT TAT GGA AGG GAT GAC TAC GTT GGG GAA GAC	1254
Tyr Lys Leu Phe Ile Tyr Gly Arg Asp Asp Tyr Val Gly Glu Asp	418
ATT GTT GAG GTC ATT AAT GAA AAC GTA AAG AAA AGC AGA AGA CTG	1299
Ile Val Glu Val Ile Asn Glu Asn Val Lys Lys Ser Arg Arg Leu	433
ATT ATC ATT TTA GTC AGA GAA ACA TCA GGC TTC AGC TGG CTG GGT	1344
Ile Ile Ile Leu Val Arg Glu Thr Ser Gly Phe Ser Trp Leu Gly	448
GGT TCA TCT GAA GAG CAA ATA GCC ATG TAT AAT GCT CTT GTT CAG	1389
Gly Ser Ser Glu Glu Gln Ile Ala Met Tyr Asn Ala Leu Val Gln	463
GAT GGA ATT AAA GTT GTC CTG CTT GAG CTG GAG AAA ATC CAA GAC	1434
Asp Gly Ile Lys Val Val Leu Leu Glu Leu Glu Lys Ile Gln Asp	478

TABLE 4C: Sequence of Coding Region of Human IL-1 Receptor Gene

TAT GAG AAA ATG CCA GAA TCG ATT AAA TTC ATT AAG CAG AAA CAT	1479
Tyr Glu Lys Met Pro Glu Ser Ile Lys Phe Ile Lys Gln Lys His	493
GGG GCT ATC CGC TGG TCA GGG GAC TTT ACA CAG GGA CCA CAG TCT	1524
Gly Ala Ile Arg Trp Ser Gly Asp Phe Thr Gln Gly Pro Gln Ser	508
GCA AAG ACA AGG TTC TGG AAG AAT GTC AGG TAC CAC ATG CCA GTC	1569
Ala Lys Thr Arg Phe Trp Lys Asn Val Arg Tyr His Met Pro Val	523
CAG CGA CGG TCA CCT TCA TCT AAA CAC CAG TTA CTG TCA CCA GCC	1614
Gln Arg Arg Ser Pro Ser Ser Lys His Gln Leu Leu Ser Pro Ala	538
ACT AAG GAG AAA CTG CAA AGA GAG GCT CAC GTG CCT CTC GGG TAG	1656
Thr Lys Glu Lys Leu Gln Arg Glu Ala His Val Pro Leu Gly End	552

CLAIMS

1. A DNA sequence consisting essentially of a single open reading frame nucleotide sequence encoding a mammalian IL-1 receptor (IL-1R) or subunit thereof.
2. A DNA sequence according to claim 1, selected from the group consisting of:
 - (a) cDNA clones having a nucleotide sequence derived from the coding region of a native mammalian IL-1R gene;
 - (b) DNA sequences capable of hybridization to the clones of (a) under moderately stringent conditions and which encode biologically active IL-1R molecules; and
 - (c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode biologically active IL-1R molecules.
3. A DNA sequence according to claim 1, consisting essentially of a synthetic gene encoding a mammalian IL-1R or subunit thereof which is capable of being expressed in a recombinant transcriptional unit comprising inducible regulatory elements derived from a microbial or viral operon.
4. A DNA sequence according to claim 3, comprising at least one sequence component derived from a cDNA sequence or copy thereof.
5. A DNA sequence according to claim 1 encoding a truncated IL-1 receptor absent a transmembrane region and cytoplasmic domain.
6. A DNA sequence according to claim 1, which is substantially similar to all or part of the sequence of nucleotides 1-1671 depicted in Tables 2A-2C.

7. A DNA sequence according to claim 1, which is substantially similar to all or part of the sequence of nucleotides 1-1656 depicted in Tables 4A-4C.

8. A recombinant expression vector comprising a DNA sequence according to any of claims 1-7.

9. A process for preparing a mammalian IL-1R or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 8 under conditions promoting expression.

10. A population of eukaryotic cells which express more than 30,000 surface IL-1 receptors per cell.

11. A population of eukaryotic cells according to claim 10, which express more than 45,000 surface IL-1 receptors per cell.

12. A homogeneous biologically active mammalian IL-1R composition.

13. A homogeneous biologically active mammalian IL-1R composition according to claim 12, consisting essentially of murine IL-1R.

14. A homogeneous biologically active mammalian IL-1R composition according to claim 12, consisting essentially of human IL-1R.

15. A protein composition comprising a soluble mammalian IL-1R, IL-1R subunit, or substantially similar or identical IL-1R analog produced by recombinant cell culture and having a specific binding activity of at least about 0.01 nanomole IL-1/nanomole IL-1R.

16. A composition according to claim 15, wherein the IL-1R composition comprises an amino acid sequence which is substantially similar to all or part of the amino acid sequence of residues 1-557 in Tables 2A-2C.

17. A composition according to claim 15, wherein the IL-1R composition comprises an amino acid sequence which is substantially similar to all or part of the amino acid sequence of residues 1-552 in Tables 4A-4C.

18. A composition according to claim 15, consisting essentially of a substantially homogeneous protein composition comprising murine IL-1 receptor in the form of a glycoprotein having a molecular weight of about 82,000 daltons by SDS-PAGE, a binding affinity (K_a) for human IL-1 α of from $3-6 \times 10^9$ M, and the N-terminal amino acid sequence NH₂-Leu-Glu-Ile-Asp-Val-Cys-Thr-Glu-Tyr-Pro-Asn-Gln-Ile-Val-Leu-Phe-Leu-Ser-Val-Asn-Glu-Ile-Asp-Ile-Arg-Lys.

19. A mammalian IL-1 receptor for use in human or veterinary medicine.

20. The use of IL-1 receptor in preparing a medicament for regulating immune or inflammatory responses in a mammal.

21. The use of claim 20, wherein the IL-1 receptor is human IL-1 receptor and the mammal to be treated is a human.

22. A composition according to claim 12 absent a transmembrane region and cytoplasmic domain.

23. A pharmaceutical composition suitable for parenteral administration to a human patient for regulating immune or inflammatory responses in mammals, comprising an effective amount of a human IL-1 receptor or biologically active human IL-1 receptor analog or subunit in admixture with a suitable diluent or carrier.

24. A process for purifying a mammalian IL-1 receptor, comprising:

(a) applying a sample comprising mammalian IL-1 receptor to an affinity matrix comprising an IL-1 or antibody molecule bound to an insoluble support; and

(b) eluting the IL-1 receptor from the affinity matrix.

25. A process according to claim 24, further comprising the steps of:

(c) applying the partially purified murine IL-1 receptor to a lectin affinity column;

(d) eluting the murine IL-1 receptor from the lectin column; and

(e) treating the partially purified murine IL-1 receptor by reversed phase high performance liquid chromatography and eluting therefrom murine IL-1 receptor as a single peak of absorbance at 280 nanometers which, when analyzed by SDS-PAGE and silver staining, appears as a single band.

26. A process according to claim 24, wherein the IL-1 molecule is recombinant human IL-1 α .

27. A method for detecting IL-1 or IL-1 receptor molecules or the interaction thereof, comprising use of a protein composition according to claim 15.

28. Antibodies immunoreactive with IL-1 receptor.

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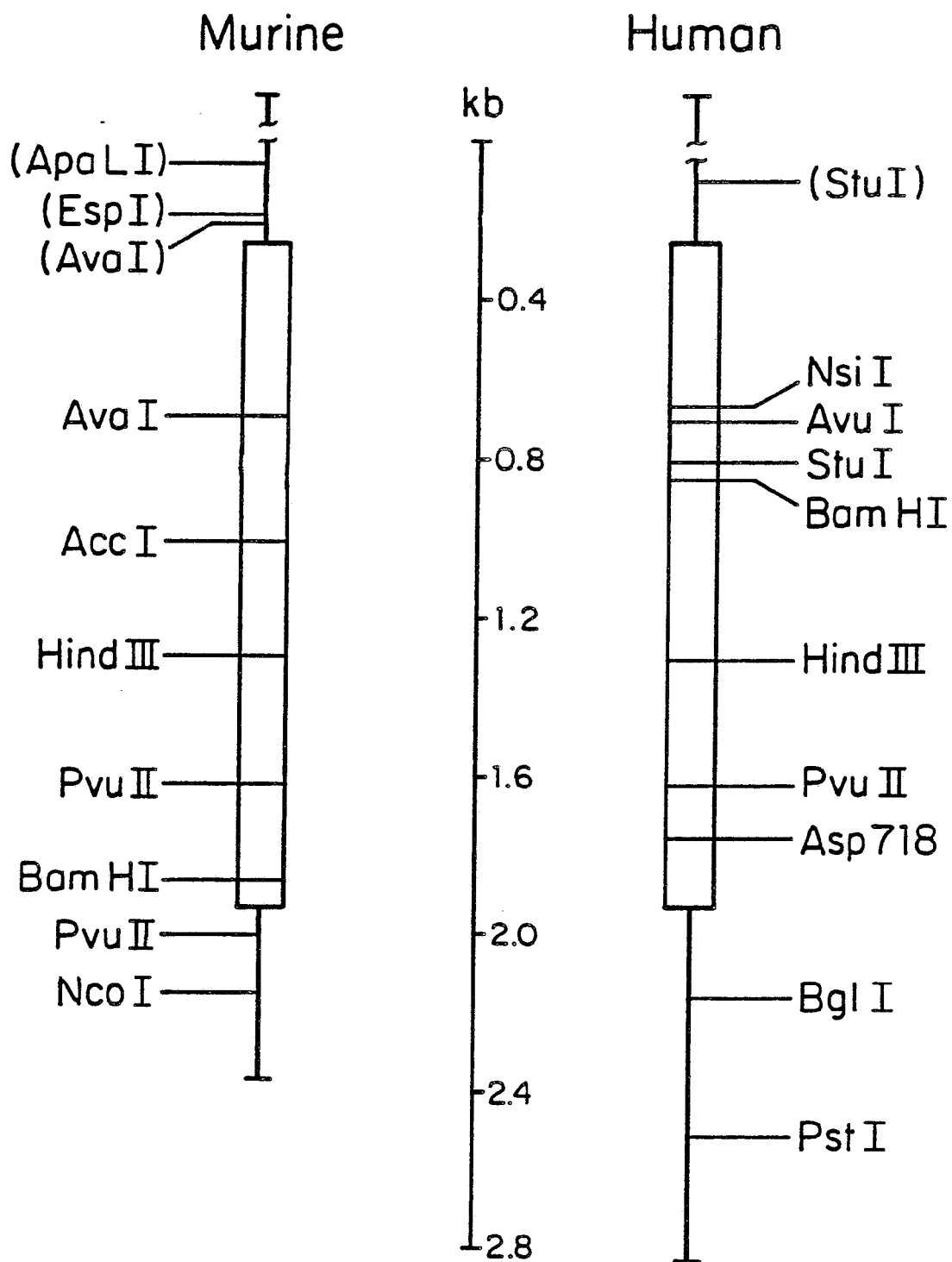


Figure 1

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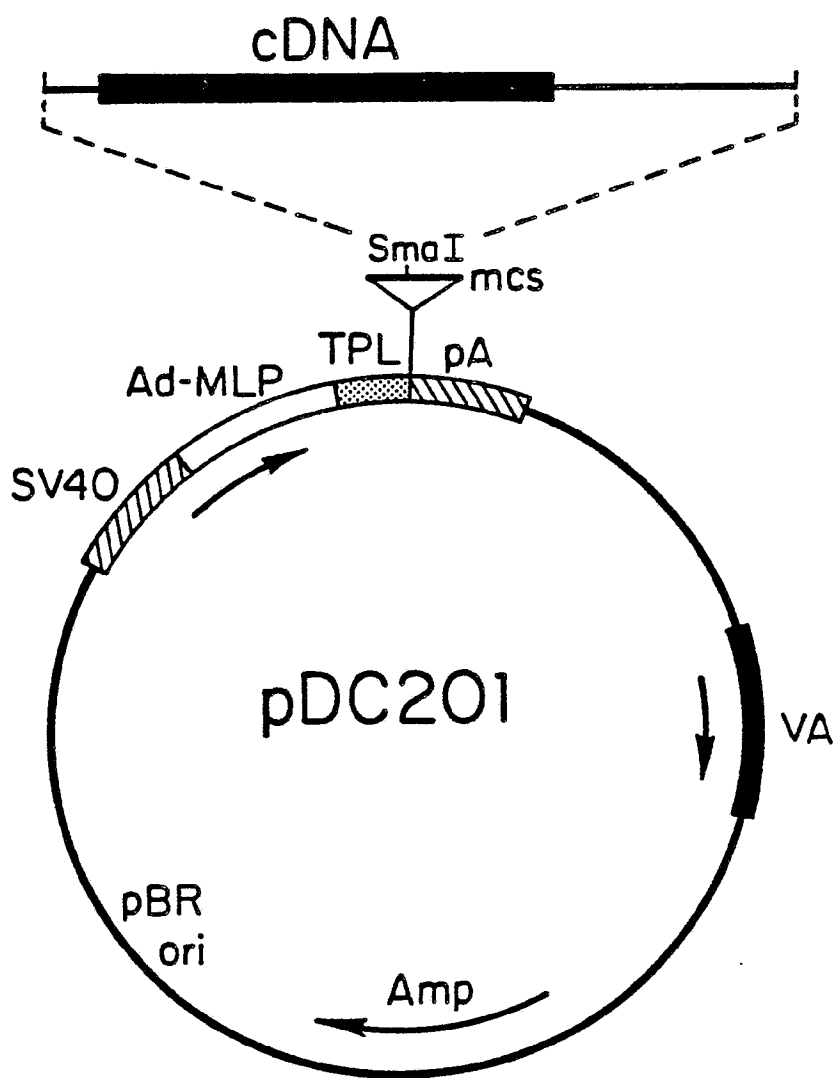


FIGURE 2

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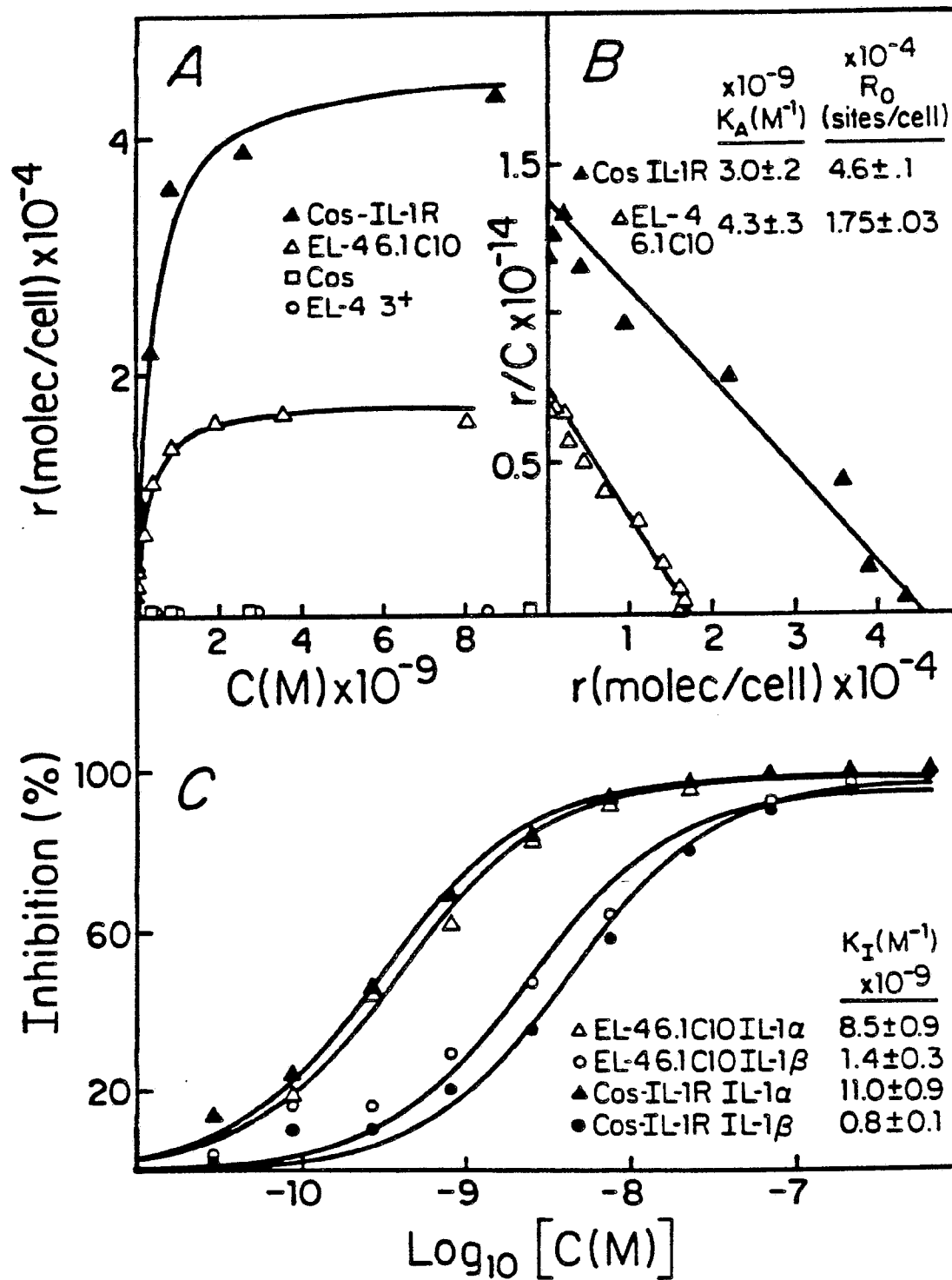


FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No.

PCT / US88/03926

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC INT. CL4 C07K 13/00,3/28; A61K37/02,39/395; C12P 21/00; C12N 15/00; C07H 19/00 U.S. CL 536/27,28; 530/350,351,395,413; 435/68, 172.3; 514/2,21;436/518;424/85.1														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; padding: 5px;">Classification System</th> <th style="border: 1px solid black; padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: middle; padding: 10px;">U. S.</td> <td style="border: 1px solid black; padding: 10px;">536/27,28;530/350,351,395,387,412,413,417,808,809 810;435/68,70,172.2,172.3,320; 424/85.1;514/2,21;885,886; 436/518;935/6,22,66,72,73</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div> <p style="padding: 5px;">Search on CAS and Dialog; Files CA, Biosis, 155,350,351; For: IL-IR and cDNA,mRNA, recombinant, purify, antibody and affinity chromatography.</p>			Classification System	Classification Symbols	U. S.	536/27,28;530/350,351,395,387,412,413,417,808,809 810;435/68,70,172.2,172.3,320; 424/85.1;514/2,21;885,886; 436/518;935/6,22,66,72,73								
Classification System	Classification Symbols													
U. S.	536/27,28;530/350,351,395,387,412,413,417,808,809 810;435/68,70,172.2,172.3,320; 424/85.1;514/2,21;885,886; 436/518;935/6,22,66,72,73													
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black; padding: 5px;">Category [*]</th> <th style="border: 1px solid black; padding: 5px;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="border: 1px solid black; padding: 5px;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 10px;"><u>X</u> <u>Y</u></td> <td style="border: 1px solid black; padding: 10px;">FEB, VOL.219, Issued July 1987,"Identification of the Plasma Membrane Receptor for Inter- lenkin-1 on Mouse Thymoma Cells." (Bron) pages 365-68, See all.</td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">12,13,15,24 1-11,16,18 20,22,25</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 10px;"><u>X</u> <u>Y</u></td> <td style="border: 1px solid black; padding: 10px;">Nature, Vol. 324, Issued November 1986, "The Cell Surface Receptor for Interlenkin- 1α and Interlenkin-1β are Identical." (Dower), pages 266-68.</td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">12-14 19 1-11,15-18, 20-28</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 10px;"><u>X</u> <u>Y</u></td> <td style="border: 1px solid black; padding: 10px;">Journal Immunology, Vol. 136, Issued June 1986, "Properties of a Specific Inter- 1(IL-1) Receptor on Human Epstein Barr Virus- Transformed B- Lymphocytes: Identity of the Receptor for IL-1α and IL-1β," (Matsushima), pages 4496-4502, See 4496-97 4505.</td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">12,14 1-11,15,17 19-28</td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	<u>X</u> <u>Y</u>	FEB, VOL.219, Issued July 1987,"Identification of the Plasma Membrane Receptor for Inter- lenkin-1 on Mouse Thymoma Cells." (Bron) pages 365-68, See all.	12,13,15,24 1-11,16,18 20,22,25	<u>X</u> <u>Y</u>	Nature, Vol. 324, Issued November 1986, "The Cell Surface Receptor for Interlenkin- 1 α and Interlenkin-1 β are Identical." (Dower), pages 266-68.	12-14 19 1-11,15-18, 20-28	<u>X</u> <u>Y</u>	Journal Immunology, Vol. 136, Issued June 1986, "Properties of a Specific Inter- 1(IL-1) Receptor on Human Epstein Barr Virus- Transformed B- Lymphocytes: Identity of the Receptor for IL-1 α and IL-1 β ," (Matsushima), pages 4496-4502, See 4496-97 4505.	12,14 1-11,15,17 19-28
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold; padding: 5px;">9 FEBRUARY 1989</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold; padding: 5px;">29 MAR 1989</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;"> International Searching Authority <div style="text-align: center; font-weight: bold; padding: 5px;">ISA/US</div> </td> <td style="border: 1px solid black; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;"> GARNETTE D. DRAFER </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold; padding: 5px;">9 FEBRUARY 1989</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold; padding: 5px;">29 MAR 1989</div>	International Searching Authority <div style="text-align: center; font-weight: bold; padding: 5px;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;"> GARNETTE D. DRAFER </div>								
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows: Group I Claims 1-11 to DNA, Vector, Cells; 536/27,435/68. Group II, claims 12-19, 22-23 to IL-1R; 530/350. Group III, claims 20-21 to methods of use, 514/21. Group IV claims 24-26 to process to purify IL-1R; 530/413. Group V, Claim 27 to detection methods; 436/518. Group VI, claim 28 to antibodies; 530/387.

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
<u>X</u> Y	J. Exp. Med., Vol 162, Issued August 1985 "Detection and Characterization of High Affinity Plasma Membrane Receptors for Human Interleukin 1," (Dower), pages 501-515, See pages 501,511-12.	<u>12,13</u> 1-11,15,16, 18-28
<u>X</u> Y	J.Exp. Med., Vol.164, Issued October 1986, " Binding and Internalization of Interleukin 1 by T-Cells," (Lowenthal), pages 1060-74, See pages, 1060,1068-72.	<u>12,13</u> 1-11,15,16 18-28
Y	US, A, 4,707,443 (Nelson), 17 November 1987, See claims.	27-28
Y	US,A, 4,578,335 (Urdal), 25 March 1987, See claims.	24-28
Y	Chemical and Engineering News, Issued August 1985," Affinity Chromatography."(Parikh), pages 17-32, See all.	24-28
Y	Journal Immunology, Vol. 136, Issued April 1986," Recombinant Human Interleukin.1: Purification and Biological Characterization." (Gubler), pages 2492-97, See all.	1-11
Y	Nature, Vol. 311, Issued October 1984 "Molecular Cloning of cDNA encoding Human Interleukin-2 Receptor,"(Nikaido), pages 631-35.	1-28
Y	Science, Vol. 234, Issued November 1986, "Novel Interleukin-2 Receptor Under High-Affinity Conditions, "(Sharon), pages 859-63.	1-28
P, A	FEB, Vol. 229, Issued February 1988,"Two-Chain Structure of the Interleukin 1 Receptor," (Kroggel), pages 59-62.	1-28

ATTACHMENT TO FORM PCT/ISA 210, Part VI

Detailed Reasons For Holding Lack of Unity of Invention:

The invention as defined by Group I (claims 1-11) is drawn to the DNA for IL-IR, vectors, processes of preparing recombinant IL-IR, and cell lines; classified in classes 536;435; and 935, subclasses 27; 68, 172.2, 320; and 6,22,and 66 respectively. Group II (claims 12-19 and 22-23) is directed to the IL-IR protein and corresponding derivatives, and pharmaceutical compositions thereto; classified in 530 and 514, subclasses 350 and 2 respectively. Group III (claims 20-21) is drawn to a therapeutic method of use of the IL-IR; classified 514, subclass 2. Group IV (claims 24-26) is directed to a method of isolating/ purifying IL-IR, classified in 530, subclass 412. Group V (claim 27) is drawn to a method of detecting IL-1 or the IL-IR, classified in 436, subclass 518. Group VI (claim 28) is drawn to an antibody, classified in 530, subclass 387.

PCT Rule 13.2 permits claims to a(one) product, a method of making the product, and a method of using the product. The invention of Groups I represents such a combination. However, the inventions of Groups II --> VI (claims 12-28) represent various products, methods of preparing the products, and different methods of use. There are no provisions in Rule 13.2 for claims covering multiple products; multiple methods of preparation; or multiple uses of the product.